1

DESCRIPTION

METHOD OF IDENTIFYING AGONIST AND ANTAGONIST

FOR TARGET PROTEIN WITH USE OF NUCLEAR

MAGNETIC RESONANCE TECHNIQUE, AND

PROGRAM FOR USE IN THE METHOD

TECHNICAL FIELD

[0001]

The present invention relates to a method of
measuring a structural variation in a protein when a
5   compound is bound to the protein by use of nuclear
magnetic resonance, a method of selecting a particular
compound based on such structural variations, and a
program for use in the methods.

BACKGROUND ART

10 [0002]

When cells respond to extracellular stimuli,
various proteins in the cells are involved in the
response. Thus, agonists and/or antagonists targeting
such proteins may be used as lead compounds for novel
15 drugs.

[0003]

Conventional methods of searching for
agonists and/or antagonists to a protein include
searching by use of physical binding to the target
20 protein as indicator and searching by use of capacity

to induce a biological reaction involving the target protein (functional induction capacities) as indicator.

[0004]

In the method of searching by use of physical binding to the target protein as indicator, compounds from a compound library (e.g., a combination library) are examined for strength of binding to the target protein, number of bound molecules per molecule thereof and so forth, and then high-affinity compounds to the target protein above a certain reference affinity are screened.

[0005]

On the other hand, in the method using functional induction capacity for the target protein, namely, a biological reaction involving the target protein, as indicator, compounds from a compound library (e.g., a combination library) are contacted with cells expressing the target protein and the resulting variations in the biological reaction (for example, qualitative variations such as initiation and termination of the biological reaction, or quantitative variations such as increased or decreased degree of the biological reaction) are examined as indicator. To carry out the method using functional induction capacity as indicator, it is required to establish an assay system specific to an individual target protein. As a consequence, when the effect of the compound is analyzed, for example, for living cells, there are such

limitations that its reliability and experimental accuracy may depend on the assay system.

[0006]

Agonists and/or antagonists to the protein are identified and the identified compounds can be used as lead compound for a novel drug. In screening candidate compounds for a novel drug, it is useful to see how the conformation of the target protein is varied by their binding to the target protein. It is because such information of conformational variations may provide a clue to the design of a candidate drug having a more appropriate structure.

[0007]

However, in either case of physical binding or functional induction capacity used as indicator, the state of binding of the compound to the protein is not directly observed. Therefore, the structure and function of the compound is speculated by analogy from the binding capacity or functional induction capacity for the target protein provided as indirect observation. Simple determination of the presence or absence of physical binding or functional induction capacity may hardly reveal the real state of interaction between the target protein and the compound and may not always provide a clear guide for drug design.

[0008]

In order to eliminate such problems presented

by conventional assay methods, an assay method
utilizing nuclear magnetic resonance (NMR) has been
published in which binding of a compound to the
functional site of a target protein is confirmed by NMR
5   (Shuker S. B. et al., Science (1996) 274, 1531-1534)
(JP-A-2001-321192). In this technique of prior art,
such a case that the compound is non-specifically bound
to a non-functional site of the target protein can be
excluded, and lead compounds can be thus identified
10 more efficiently than in the conventional assay of
physical binding which has a difficulty in
distinguishing between nonspecific binding and specific
binding. However, the technique is not suitable to
measure a structural variation itself in the protein,
15 though it enables the binding site to be identified.
Therefore, this technique is effective to design
inhibitors capable of binding to the active site of the
target protein, but is not so effective to design
agonistic agents (e.g., agonists) capable of inducing
20 the activity of the protein. Since identification of
an agonistic lead compound requires to screen compounds
of interest by seeing if they bind properly to the
binding site on the target protein to its substrate and
further induce the active structure of the target
25 protein, this conventional technique has not been
adequate to identify the agonistic lead compound.
[0009]

     It can be determined by structural analysis

based on X-ray crystallography if a compound can induce the active structure of a target protein. However, the X-ray crystallographical structural analysis is not suitable for efficient screening since it needs

5 enormous amounts of labor and time to crystallize many compounds together with the target protein and analyze the conformation of as many crystals. Consequently, a simple and rapid method to determine a structural change in the target protein, which may occur when an

10 arbitrary compound is contacted with the target protein and bound thereto, and a computer program to carry out the method are needed.

Patent Document 1: JP-A-2001-321192.

Non-Patent Document 1: Shuker S. B. et al.,

15 Science (1996) 274, 1531-1534.

DISCLOSURE OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0010]

The subject of the present invention is to

20 provide a simple and rapid method of determining a structural change in the target protein, which may occur when an arbitrary compound is contacted with the target protein and bound thereto, a method of screening compounds for selection by using the above mentioned

25 method and a computer program to carry out those methods.

[0011]

It is one subject of the present invention to provide a method of measuring a structural change in a protein, which may occur when an arbitrary compound is contacted with the protein and bound thereto, for

5   example, using nuclear magnetic resonance. It is also one subject of the present invention to provide a method of identifying an arbitrary compound for screening which may induce a structural change in a protein when the compound is contacted with the protein

10 and bound thereto. It is another subject of the present invention to provide a method of identifying a compound for screening which induces a structural change in a protein similar to that induced by a ligand or an agonist, by comparing a structural change in the

15 protein when an arbitrary compound is bound thereto with a structural change in the same protein when such a compound as a ligand or an agonist is bound thereto, which is known to induce a structural change in a protein when it is bound to the protein.

20 [0012]

It is a further subject of the present invention to provide a computer program used in these methods.

MEANS FOR SOLVING THE PROBLEMS

25 [0013]

The present inventors have developed a method of estimating a conformational change in a target

protein, which is induced by addition of a compound
into a solution of the target protein, by a change
thereof in a TROSY correlation NMR spectrum (for
example, ^{1}H/^{15}N NMR spectrum) and a program therefor,
5 and completed the present invention thereby. The
present inventors have developed a novel method capable
of concurrent observation of both of 1) a site on a
target protein to which a compound is bound, and 2) a
structural change in the target protein induced by the
10 compound, resulting in the completion of the invention.
[0014]

       Thus, the present invention provides the
following:

       1. A method of measuring a structural change
15 in a protein when the protein is contacted with a
compound, comprising the steps of:

       (a) selecting a domain in the protein;

       (b) providing information on an orientation
of the domain when the protein is not in contact with
20 the compound;

       (c) providing information on an orientation
of the domain when the protein is in contact with the
compound, by

       (i) providing known atomic coordinates for
25 the domain,

       (ii) providing axial variations of NMR
signals, which are generated from the protein in
contact with the compound in the presence of a liquid

crystalline material by two-dimensional TROSY NMR spectroscopy and depend on an orientation angle of the molecules in a magnetic field,

(iii) determining Saupe order matrix elements for the domain from the atomic coordinates for the domain and the axial variations of NMR signals, and

(iv) diagonalizing the determined matrix to produce the information on an orientation of the domain; and

(d) measuring the structural change in the protein by a difference between the information on an orientation provided in step (b) and the information on an orientation provided in step (c);

2. The method of measurement according to item 1, wherein the step (b) is a step of:

(b) providing the information on an orientation of the domain when the protein is not in contact with the compound, by

(v) providing known atomic coordinates for the domain,

(vi) providing axial variations of NMR signals, which are generated from the protein in no contact with the compound in the presence of the liquid crystalline material by two-dimensional TROSY NMR spectroscopy and depend on the orientation angle of the molecules in the magnetic field,

(vii) determining Saupe order matrix elements for the domain from the atomic coordinates for the

domain and the axial variations of NMR signals, and

(viii) diagonalizing the determined matrix to produce the information on an orientation of the domain;

5   3. The method of measurement according to item 1, wherein the step (b) is a step of

(b) providing the information on an orientation of the domain from the atomic coordinates provided previously when the protein is not in contact

10   with the compound;

4. The method of measurement according to item 1, wherein in the step (c), the axial variations of NMR signals, which are generated by two-dimensional TROSY NMR spectroscopy and depend on the orientation

15   angle of the molecules in the magnetic field, are variations along a ^{15}N axis;

5. The method of measurement according to item 4, wherein the Saupe order matrix elements in (iii) are determined by:

20   with respect to the kth pair of ^{15}N nuclear spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond having the kth pair of spins in the domain to the ith molecular axis,

25   providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth molecular axis,

setting a static dipolar coupling D^0_{nh} at 23.0

kHz for a length of the N-H bond of 1.02 Å, or setting

a static dipolar coupling D^0_{nh} at 21.7 kHz for a length

of the N-H bond of 1.04 Å,

determining as δ_{ij} a tensor value for chemical

shift anisotropy of a ^{15}N nucleus in an arbitrary

residue of the protein, and

determining the Saupe order matrix elements

S_{ij} defining the molecular orientation with respect to

the magnetic field, by contacting the protein with the

compound in the presence of the liquid crystalline

material, providing $\Delta\delta$trosy(k) for the kth pair of ^{15}N

nuclear spins by two-dimensional TROSY NMR

spectroscopy, and using the $\Delta\delta$trosy(k) together with the

following equation (1):

$\Delta\delta$trosy(k) $= \sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j +(2/3)\ \delta_{ij}\}$ (1)

i, j = x, y, z;

6. The method of measurement according to

item 2, wherein in the step (b), the axial variations

of NMR signals, which are generated by two-dimensional

TROSY NMR spectroscopy and depend on the orientation

angle of the molecules in the magnetic field, are

variations along a ^{15}N axis;

7. The method of measurement according to

item 6, wherein the Saupe order matrix elements in

(vii) are determined by:

with respect to the kth pair of ^{15}N nuclear

spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond

having the kth pair of spins in the domain to the ith

molecular axis,

$\quad$ providing ϕ^k_j as a vector angle of an N-H bond

having the kth pair of spins in the domain to the jth

5 $\quad$ molecular axis,

$\quad$ setting a static dipolar coupling D^0_{nh} at 23.0

kHz for a length of the N-H bond of 1.02 Å, or setting

a static dipolar coupling D^0_{nh} at 21.7 kHz for a length

of the N-H bond of 1.04 Å,

10 $\quad$ determining as δ_{ij} a tensor value for chemical

shift anisotropy of a ^{15}N nucleus in an arbitrary

residue of the protein, and

$\quad$ determining the Saupe order matrix elements

S_{ij} defining the molecular orientation with respect to

15 $\quad$ the magnetic field, by making no contact of the protein

with the compound in the presence of the liquid

crystalline material, providing $\Delta\delta$trosy(k) for the kth

pair of ^{15}N nuclear spins by two-dimensional TROSY NMR

spectroscopy, and using the $\Delta\delta$trosy(k) together with the

20 $\quad$ following equation (1):

$\Delta\delta$trosy(k) = $\sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j + (2/3)\ \delta_{ij}\}$ $\quad$ (1)

$\quad\quad$ i, j = x, y, z;

$\quad$ 8. The method according to item 5 or 7,

wherein a structural change in the protein when the

25 $\quad$ protein and the compound are contacted is digitized as

degree of orientational change by:

$\quad$ (ix) providing directions of orientation

tensor axes by a first three unit vectors perpendicular

to each other, by using the information on an orientation of the domain in the protein before the protein is contacted with the compound, wherein the first three unit vectors are expressed by

5 [0015]

$$\overrightarrow{e}_{fx}, \quad \overrightarrow{e}_{fy}, \quad \overrightarrow{e}_{fz}$$

(x) providing directions of orientation tensor axes by a second three unit vectors perpendicular to each other, by using the information on an orientation of the domain in the protein after

10 the protein is contacted with the compound, wherein the second three unit vectors are expressed by

[0016]

$$\overrightarrow{e}_{bx}, \quad \overrightarrow{e}_{by}, \quad \overrightarrow{e}_{bz}$$

(xi) denoting respective angles between the individual first unit vectors and the individual second

15 unit vectors by a, b and c, and

(xii) giving a degree of orientational change by the following equation:

degree of orientational change $= a^2 + b^2 + c^2$;

9. The method according to item 2, further

20 comprising a step of identifying a position on the protein to which the compound is bound;

10. The method according to item 9, wherein

the step of identifying a position on the protein to which the compound is bound is carried out by comparing the two-dimensional TROSY NMR spectrum obtained in the step (b) with the two-dimensional TROSY NMR spectrum

5 obtained in the step (c) to detect a spectral change, and identifying an amino acid residue in the protein which has induced the spectral change;

11. The method according to item 1, wherein the liquid crystalline material comprises a mixture

10 selected from the following group:

a mixture of dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcholine (DHPC),

a mixture of dimyristoylphosphatidylcholine (DMPC), dihexanoylphosphatidylcholine (DHPC), and

15 sodium dodecyl sulfate (SDS),

a mixture of dimyristoylphosphatidylcholine (DMPC), dihexanoylphosphatidylcholine (DHPC), and cetyltrimethylammonium bromide (CTAB),

a mixture of 1,2-di-O-dodecyl-sn-glycero-3-

20 phosphocholine (DIODPC) and 3-(cholamidepropyl)-dimethylammonio-2-hydroxy-1-propane sulfate (CHAPS),

a mixture of n-alkyl-poly(ethyleneglycol)/n-alkylalcohol,

filamentous phage,

25 a mixture of cetylpyridinium chloride (CPCl) - hexanol - NaCl,

a mixture of cetylpyridinium bromide (CPBr) - hexanol - NaCl,

a purple membrane fragment of Halobacterium
spp.,

microcrystalline cellulose, and

polyacrylamide gel;

5   12. The method according to item 11, wherein
the liquid crystalline material is the mixture of 7.5%
(w/v) composed of dimyristoylphosphatidylcholine
(DMPC), dihexanoylphosphatidylcholine (DHPC), and
cetyltrimethylammonium bromide (CTAB);

10   13. A method of selecting a compound capable
of inducing a structural change in a domain within a
protein when the protein is contacted with the
compound, comprising the steps of:

            (a) selecting a domain in the protein;

15   (b) providing information on an orientation
of the domain when the protein is not in contact with
the compound;

            (c) providing information on an orientation
of the domain when the protein is in contact with the

20   compound, by

            (i) providing known atomic coordinates for
the domain,

            (ii) providing axial variations of NMR
signals, which are generated from the protein in

25   contact with the compound in the presence of a liquid
crystalline material by two-dimensional TROSY NMR
spectroscopy and depend on an orientation angle of the
molecules in a magnetic field,

(iii) determining Saupe order matrix elements for the domain from the atomic coordinates for the domain and the axial variations of NMR signals, and

(iv) diagonalizing the determined matrix to produce the information on an orientation of the domain; and

(d) determining if the compound is capable of inducing a structural change in the domain when the protein is contacted with the compound, by making a comparison between the information on an orientation provided in step (b) and the information on an orientation provided in step (c);

14. The method according to item 13, wherein the step (b) is a step of:

(b) providing the information on an orientation of the domain when the protein is not in contact with the compound, by

(v) providing known atomic coordinates for the domain,

(vi) providing axial variations of NMR signals, which are generated from the protein in no contact with the compound in the presence of the liquid crystalline material by two-dimensional TROSY NMR spectroscopy and depend on the orientation angle of the molecules in the magnetic field,

(vii) determining Saupe order matrix elements for the domain from the atomic coordinates for the domain and the axial variations of NMR signals, and

(viii) diagonalizing the determined matrix to produce the information on an orientation of the domain;

15. The method according to item 13, wherein the step (b) is a step of

(b) providing the information on an orientation of the domain from the atomic coordinates provided previously when the protein was not in contact with the compound;

16. The method according to item 13, wherein in the step (c), the axial variations of NMR signals, which are generated by two-dimensional TROSY NMR spectroscopy and depend on the orientation angle of the molecules in the magnetic field, are variations along a ^{15}N axis;

17. The method according to item 16, wherein the Saupe order matrix elements in (iii) are determined by:

with respect to the kth pair of ^{15}N nuclear spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond having the kth pair of spins in the domain to the ith molecular axis,

providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth molecular axis,

setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N-H bond of 1.02 Å, or setting

a static dipolar coupling D^0_{nh} at 21.7 kHz for a length
of the N-H bond of 1.04 Å

determining as δ_{ij} a tensor value for chemical
shift anisotropy of a ^{15}N nucleus in an arbitrary
5 residue of the protein, and

determining the Saupe order matrix elements
S_{ij} defining the molecular orientation with respect to
the magnetic field, by contacting the protein with the
compound in the presence of the liquid crystalline
10 material, providing $\Delta\delta trosy(k)$ for the kth pair of ^{15}N
nuclear spins by two-dimensional TROSY NMR
spectroscopy, and using the $\Delta\delta trosy(k)$ together with the
following equation (1):

$$\Delta\delta trosy(k) = \sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j + (2/3)\ \delta_{ij}\} \quad (1)$$
15       $i, j = x, y, z;$

18. The method according to item 14, wherein
in the step (b), the axial variations of NMR signals,
which are generated by two-dimensional TROSY NMR
spectroscopy and depend on the orientation angle of the
20 molecules in the magnetic field, are variations along a
^{15}N axis;

19. The method according to item 18, wherein
the Saupe order matrix elements in (vii) are determined
by:

25   with respect to the kth pair of ^{15}N nuclear
spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond
having the kth pair of spins in the domain to the ith

molecular axis,

providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth molecular axis,

5 setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N-H bond of 1.02 Å, or setting a static dipolar coupling D^0_{nh} at 21.7 kHz for a length of the N-H bond of 1.04 Å

determining as δ_{ij} a tensor value for chemical
10 shift anisotropy of a ^{15}N nucleus in an arbitrary residue of the protein, and

determining the Saupe order matrix elements S_{ij} defining the molecular orientation with respect to the magnetic field, by making no contact of the protein
15 with the compound in the presence of the liquid crystalline material, providing Δδtrosy(k) for the kth pair of ^{15}N nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the Δδtrosy(k) together with the following equation (1):

20 $\Delta\delta trosy(k) = \sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j + (2/3)\ \delta_{ij}\}$ (1)

$i, j = x, y, z;$

20. The method according to item 17 or 19, wherein the comparison with respect to the information on an orientation in the step (c) is carried out by:

25 (ix) providing directions of orientation tensor axes by a first three unit vectors perpendicular to each other, by using the information on an orientation of the domain in the protein before the

protein is contacted with the compound, wherein the
first three unit vectors are expressed by
[0017]

$$\vec{e}_{fx}, \quad \vec{e}_{fy}, \quad \vec{e}_{fz}$$

(x) providing directions of orientation
5 tensor axes by a second three unit vectors
perpendicular to each other, by using the information
on an orientation of the domain in the protein after
the protein is contacted with the compound, wherein the
second three unit vectors are expressed by
10 [0018]

$$\vec{e}_{bx}, \quad \vec{e}_{by}, \quad \vec{e}_{bz}$$

(xi) denoting respective angles between the
individual first unit vectors and the individual second
unit vectors by a, b and c,
(xii) giving a degree of orientational change
15 by the following equation:
degree of orientational change = $a^2 + b^2 + c^2$
wherein the degree of orientational change is
compared;
21. The method according to item 14, further
20 comprising a step of identifying a position on the
protein to which the compound is bound;
22. The method according to item 21, wherein

the step of identifying a position on the protein to
which the compound is bound is carried out by comparing
the two-dimensional TROSY NMR spectrum obtained in the
step (b) with the two-dimensional TROSY NMR spectrum

5   obtained in the step (c) to detect a spectral change,
and identifying an amino acid residue which has induced
the spectral change;

   23. The method according to item 13, wherein
the liquid crystalline material comprises a mixture

10 selected from the group consisting of:

   a mixture of dimyristoylphosphatidylcholine
(DMPC) and dihexanoylphosphatidylcholine (DHPC),

   a mixture of dimyristoylphosphatidylcholine
(DMPC), dihexanoylphosphatidylcholine (DHPC), and

15 sodium dodecyl sulfate (SDS),

   a mixture of dimyristoylphosphatidylcholine
(DMPC), dihexanoylphosphatidylcholine (DHPC), and
cetyltrimethylammonium bromide (CTAB),

   a mixture of 1,2-di-O-dodecyl-sn-glycero-3-

20 phosphocholine (DIODPC) and 3-(cholamidepropyl)-
dimethylammonio-2-hydroxy-1-propane sulfate (CHAPS),

   a mixture of n-alkyl-poly(ethyleneglycol)/n-
alkylalcohol,

   filamentous phage,

25   a mixture of cetylpyridinium chloride (CPCl)
- hexanol - NaCl,

   a mixture of cetylpyridinium bromide (CPBr) -
hexanol - NaCl,

a purple membrane fragment of Halobacterium

spp.,

microcrystalline cellulose, and

polyacrylamide gel;

5   24. The method according to item 23, wherein

the liquid crystalline material is the mixture of

7.5%(w/v) composed of dimyristoylphosphatidylcholine

(DMPC), dihexanoylphosphatidylcholine (DHPC), and

cetyltrimethylammonium bromide (CTAB);

10   25. A method of selecting a second compound

capable of inducing a structural change in a protein on

contact with the protein, which is similar to a

structural change in the protein induced by a first

compound on contact with the protein, comprising the

15   steps of:

(a) selecting a domain in the protein;

(b) providing information on an orientation

of the domain when the protein is in contact with the

first compound;

20   (c) providing information on an orientation

of the domain when the protein is in contact with the

second compound, by

(i) providing known atomic coordinates for

the domain,

25   (ii) providing axial variations of NMR

signals, which are generated from the protein in

contact with the second compound in the presence of a

liquid crystalline material by two-dimensional TROSY

NMR spectroscopy and depend on an orientation angle of the molecules in a magnetic field,

(iii) determining Saupe order matrix elements for the domain from the atomic coordinates for the domain and the axial variations of NMR signals, and

(iv) diagonalizing the determined matrix to produce the information on an orientation of the domain;

(d) making a comparison between the information on an orientation provided in step (b) and the information on an orientation provided in step (c); and

(e) determining from the results obtained by the comparison in step (d) if the structural change in the protein induced by the second compound on contact with the protein is similar to the structural change in the protein induced by the first compound on contact with the protein;

26. The method according to item 25, wherein the step (b) is a step of:

(b) providing the information on an orientation of the domain when the protein is in contact with the first compound, by

(v) providing known atomic coordinates for the domain,

(vi) providing axial variations of NMR signals, which are generated from the protein in contact with the first compound in the presence of the

liquid crystalline material by two-dimensional TROSY

NMR spectroscopy and depend on an orientation angle of

the molecules in the magnetic field,

      (vii) determining Saupe order matrix elements

for the domain from the atomic coordinates for the

domain and the axial variations of NMR signals, and

      (viii) diagonalizing the determined matrix to

produce the information on an orientation of the

domain;

      27. The method according to item 25, wherein

the step (b) is a step of

      (b) providing the information on an

orientation of the domain from the atomic coordinates

provided previously when the protein was in contact

with the first compound;

      28. The method according to item 25, wherein

in the step (c), the axial variations of NMR signals,

which are generated by two-dimensional TROSY NMR

spectroscopy and depend on the orientation angle of the

molecules in the magnetic field, are variations along a

^{15}N axis;

      29. The method according to item 28, wherein

the Saupe order matrix elements in (iii) are determined

by:

      with respect to the kth pair of ^{15}N nuclear

spins in the domain,

      providing ϕ^k_i as a vector angle of an N-H bond

having the kth pair of spins in the domain to the ith

molecular axis,

providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth molecular axis,

5 setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N-H bond of 1.02 Å, or setting a static dipolar coupling D^0_{nh} at 21.7 kHz for a length of the N-H bond of 1.04 Å,

determining as δ_{ij} a tensor value for chemical

10 shift anisotropy of a ^{15}N nucleus in an arbitrary residue of the protein, and

determining the Saupe order matrix elements S_{ij} defining the molecular orientation with respect to the magnetic field, by contacting the protein with the

15 compound in the presence of the liquid crystalline material, providing $\Delta\delta$trosy(k) for the kth pair of ^{15}N nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the $\Delta\delta$trosy(k) together with the following equation (1):

20 $\Delta\delta$trosy(k) $= \sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j + (2/3)\ \delta_{ij}\}$ (1)

i, j = x, y, z;

30. The method according to item 26, wherein in the step (b), the axial variations of NMR signals, which are generated by two-dimensional TROSY NMR

25 spectroscopy and depend on the orientation angle of the molecules in the magnetic field, are variations along a ^{15}N axis;

31. The method according to item 30, wherein

the Saupe order matrix elements in (vii) are determined by:

with respect to the kth pair of ^{15}N nuclear spins in the domain,

5   providing ϕ^k_i as a vector angle of an N-H bond having the kth pair of spins in the domain to the ith molecular axis,

providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth

10   molecular axis,

setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N-H bond of 1.02 Å, or setting a static dipolar coupling D^0_{nh} at 21.7 kHz for a length of the N-H bond of 1.04 Å,

15   determining as δ_{ij} a tensor value for chemical shift anisotropy of a ^{15}N nucleus in an arbitrary residue of the protein, and

determining the Saupe order matrix elements S_{ij} defining the molecular orientation with respect to

20   the magnetic field, by contacting the protein with the second compound in the presence of the liquid crystalline material, providing $\Delta\delta$trosy(k) for the kth pair of ^{15}N nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the

25    $\Delta\delta$trosy(k) together with the following equation (1):

$$\Delta\delta\text{trosy}(k) = \sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j + (2/3)\ \delta_{ij}\} \qquad (1)$$

i, j = x, y, z;

32. The method according to item 29 or 31,

wherein the comparison with respect to the information on an orientation in the step (d) is carried out by:

(ix) providing directions of orientation tensor axes by a first three unit vectors perpendicular to each other, by using the information on an orientation of the domain in the protein when the protein is contacted with the first compound, wherein the first three unit vectors are expressed by

[0019]

$$\overrightarrow{e}_{ax}, \quad \overrightarrow{e}_{ay}, \quad \overrightarrow{e}_{az}$$

(x) providing directions of orientation tensor axes by a second three unit vectors perpendicular to each other, by using the information on an orientation of the domain in the protein when the protein is contacted with the second compound, wherein the second three unit vectors are expressed by

[0020]

$$\overrightarrow{e}_{bx}, \quad \overrightarrow{e}_{by}, \quad \overrightarrow{e}_{bz}$$

(xi) denoting respective angles between the individual first unit vectors and the individual second unit vectors by a, b and c and using the a, b and c as indices for the comparison with respect to the information on an orientation;

33. The method according to item 29 or 31,

wherein the comparison with respect to the information on an orientation in the step (d) is carried out by:

(ix) providing directions of orientation tensor axes by a first three unit vectors perpendicular to each other, by using the information on an orientation of the domain in the protein when the protein is contacted with the first compound, wherein the first three unit vectors are expressed by

[0021]

$$\overrightarrow{e}_{ax}, \quad \overrightarrow{e}_{ay}, \quad \overrightarrow{e}_{az}$$

(x) providing directions of orientation tensor axes by a second three unit vectors perpendicular to each other, by using the information on an orientation of the domain in the protein when the protein is contacted with the second compound, wherein the second three unit vectors are expressed by

[0022]

$$\overrightarrow{e}_{bx}, \quad \overrightarrow{e}_{by}, \quad \overrightarrow{e}_{bz}$$

(xi) denoting respective angles between the individual first unit vectors and the individual second unit vectors by a, b and c, and

(xii) giving a degree of orientational similarity to the active conformation by the following equation:

degree of orientational similarity to the

active conformation = $a^2 + b^2 + c^2$

wherein the degree of orientational

similarity to the active conformation is used as index

5 for the comparison with respect to the information on

an orientation;

34. The method according to item 25, further

comprising the step of identifying a position on the

protein to which at least one of the first and second

10 compounds is bound;

35. The method according to item 34, wherein

the step of identifying a position on the protein to

which at least one of the first and second compounds is

bound is carried out by comparing a two-dimensional

15 TROSY NMR spectrum obtained in the absence of the

compound with a two-dimensional TROSY NMR spectrum

obtained in the presence of the compound to detect a

spectral change, and identifying an amino acid residue

which has induced the spectral change;

20 36. The method according to item 25, wherein

the liquid crystalline material comprises a mixture

selected from the group consisting of:

a mixture of dimyristoylphosphatidylcholine

(DMPC) and dihexanoylphosphatidylcholine (DHPC),

25 a mixture of dimyristoylphosphatidylcholine

(DMPC), dihexanoylphosphatidylcholine (DHPC), and

sodium dodecyl sulfate (SDS),

a mixture of dimyristoylphosphatidylcholine

(DMPC), dihexanoylphosphatidylcholine (DHPC), and
cetyltrimethylammonium bromide (CTAB),

a mixture of 1,2-di-O-dodecyl-sn-glycero-3-phosphocholine (DIODPC) and 3-(cholamidepropyl)-

5 dimethylammonio-2-hydroxy-1-propane sulfate (CHAPS),

a mixture of n-alkyl-poly(ethyleneglycol)/n-alkylalcohol,

filamentous phage,

a mixture of cetylpyridinium chloride (CPCl)

10 - hexanol - NaCl,

a mixture of cetylpyridinium bromide (CPBr) - hexanol - NaCl,

a purple membrane fragment of Halobacterium spp.,

15 microcrystalline cellulose, and

polyacrylamide gel;

37. The method according to item 36 wherein the liquid crystalline material is the mixture of 7.5%(w/v) composed of dimyristoylphosphatidylcholine

20 (DMPC), dihexanoylphosphatidylcholine (DHPC), and cetyltrimethylammonium bromide (CTAB);

38. A program of digitizing a structural change in a selected domain within a protein when the protein is contacted with a compound, comprising

25 carrying out on a computer the means for:

(a) providing information on an orientation of the domain when the protein is not in contact with the compound;

(b) providing information on an orientation of the domain when the protein is in contact with the compound, by

(i) providing data on known atomic

5 coordinates for the domain,

(ii) providing data on axial variations of NMR signals, which are generated from the protein in contact with the compound in the presence of a liquid crystalline material by two-dimensional TROSY NMR

10 spectroscopy and depend on an orientation angle of the molecules in a magnetic field,

(iii) determining Saupe order matrix elements for the domain from the atomic coordinates for the domain and the axial variations of NMR signals, and

15 (iv) diagonalizing the determined matrix to produce the information on an orientation of the domain; and

(c) calculating the structural change in the protein by a difference between the information on an

20 orientation provided in means (a) and the information on an orientation provided in means (b);

39. The program according to item 38, wherein the means (a) is means for

(a) providing information on an orientation

25 of the domain when the protein is not in contact with the compound, by

(v) providing the known atomic coordinates for the domain,

(vi) providing axial variations of NMR signals, which are generated from the protein in no contact with the compound in the presence of the liquid crystalline material by two-dimensional TROSY NMR

5 spectroscopy and depend on an orientation angle of the molecules in the magnetic field,

(vii) determining Saupe order matrix elements for the domain from the atomic coordinates for the domain and the axial variations of NMR signals, and

10 (viii) diagonalizing the determined matrix to produce the information on an orientation of the domain;

40. The program according to item 38, wherein the means (a) is means for

15 (a) providing the information on an orientation of the domain from the atomic coordinates provided previously when the protein was not in contact with the compound;

41. The program according to item 38, wherein

20 in the step (b), the axial variations of NMR signals, which are generated by two-dimensional TROSY NMR spectroscopy and depend on the orientation angle of the molecules in the magnetic field, are variations along a ^{15}N axis;

25 42. The program according to item 41, wherein the Saupe order matrix elements in (iii) are determined by:

with respect to the kth pair of ^{15}N nuclear

spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond having the kth pair of spins in the domain to the ith molecular axis,

5 providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth molecular axis,

setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N-H bond of 1.02 Å, or setting

10 a static dipolar coupling D^0_{nh} at 21.7 kHz for a length of the N-H bond of 1.04 Å,

determining as δ_{ij} a tensor value for chemical shift anisotropy of a ^{15}N nucleus in an arbitrary residue of the protein, and

15 determining the Saupe order matrix elements S_{ij} defining the molecular orientation with respect to the magnetic field, by contacting the protein with the compound in the presence of the liquid crystalline material, providing Δδtrosy(k) for the kth pair of ^{15}N

20 nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the Δδtrosy(k) together with the following equation (1):

$$\Delta\delta\text{trosy}(k) = \sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j + (2/3)\ \delta_{ij}\} \qquad (1)$$

$$i,\ j = x,\ y,\ z;$$

25 43. The program according to item 39, wherein in the step (a), the axial variations of NMR signals, which are generated by two-dimensional TROSY NMR spectroscopy and depend on the orientation angle of the

molecules in the magnetic field, are variations along a ^{15}N axis;

44. The program according to item 43, wherein the Saupe order matrix elements in (vii) are determined by:

with respect to the kth pair of ^{15}N nuclear spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond having the kth pair of spins in the domain to the ith molecular axis,

providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth molecular axis,

setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N-H bond of 1.02 Å, or setting a static dipolar coupling D^0_{nh} at 21.7 kHz for a length of the N-H bond of 1.04 Å,

determining as δ_{ij} a tensor value for chemical shift anisotropy of a ^{15}N nucleus in an arbitrary residue of the protein, and

determining the Saupe order matrix elements S_{ij} defining the molecular orientation with respect to the magnetic field, by making no contact of the protein with the compound in the presence of the liquid crystalline material, providing $\Delta\delta$trosy(k) for the kth pair of ^{15}N nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the $\Delta\delta$trosy(k) together with the following equation (1):

$$\Delta\delta trosy(k) = \sum S_{ij}\{0.5\ D^0_{nh}\cos\phi^k_i\cos\phi^k_j + (2/3)\ \delta_{ij}\} \qquad (1)$$

$$i,\ j = x,\ y,\ z;$$

45. The program according to item 42 or 44, wherein a structural change in the protein when the protein and the compound are contacted is digitized as degree of orientational change by:

(ix) providing directions of orientation tensor axes by a first three unit vectors perpendicular to each other, by using the information on an orientation of the domain in the protein before the protein is contacted with the compound, wherein the first three unit vectors are expressed by

[0023]

$$\vec{e}_{fx},\ \vec{e}_{fy},\ \vec{e}_{fz}$$

(x) providing directions of orientation tensor axes by a second three unit vectors perpendicular to each other, by using the information on an orientation of the domain in the protein after the protein is contacted with the compound, wherein the second three unit vectors are expressed by

[0024]

$$\vec{e}_{bx},\ \vec{e}_{by},\ \vec{e}_{bz}$$

(xi) denoting respective angles between the individual first unit vectors and the individual second

unit vectors by a, b and c,

(xii) giving a degree of orientational change by the following equation:

degree of orientational change = $a^2 + b^2 + c^2$;

5   46. The program according to item 39, further comprising means for identifying a position on the protein to which the compound is bound;

47. The program according to item 46, wherein the means of identifying a position on the protein to 10 which the compound is bound is carried out by comparing the two-dimensional TROSY NMR spectrum obtained in the step (a) with the two-dimensional TROSY NMR spectrum obtained in the step (b) to detect a spectral change, and identifying an amino acid residue in the protein 15 which has induced the spectral change;

48. A program of selecting a compound capable of inducing a structural change in a domain within a protein when the protein is contacted with the compound, comprising the means of:

20   (a) providing information on an orientation of the domain when the protein is not in contact with the compound;

(b) providing information on an orientation of the domain when the protein is in contact with the 25 compound, by

(i) providing known atomic coordinates for the domain,

(ii) providing axial variations of NMR

signals, which are generated from the protein in contact with the compound in the presence of a liquid crystalline material by two-dimensional TROSY NMR spectroscopy and depend on an orientation angle of the

5　molecules in a magnetic field,

(iii) determining Saupe order matrix elements for the domain from the atomic coordinates for the domain and the axial variations of NMR signals, and

(iv) diagonalizing the determined matrix to

10　produce the information on an orientation of the domain; and

(c) determining if the compound is capable of inducing a structural change in the domain when the protein is contacted with the compound, by making a

15　comparison between the information on an orientation provided in step (a) and the information on an orientation provided in step (b);

49. The program according to item 48, wherein the means (a) is means for

20　　　　　(a) providing information on an orientation of the domain when the protein is not in contact with the compound, by

(v) providing the known atomic coordinates for the domain,

25　　　　　(vi) providing axial variations of NMR signals, which are generated from the protein in no contact with the compound in the presence of the liquid crystalline material by two-dimensional TROSY NMR

spectroscopy and depend on an orientation angle of the molecules in the magnetic field,

(vii) determining Saupe order matrix elements for the domain from the atomic coordinates for the

5   domain and the axial variations of NMR signals, and

(viii) diagonalizing the determined matrix to produce the information on an orientation of the domain;

50. The program according to item 48, wherein

10 the means (a) is means for

(a) providing the information on an orientation of the domain from the atomic coordinates provided previously when the protein was not in contact with the compound;

15   51. The program according to item 48, wherein in the step (b), the axial variations of NMR signals, which are generated by two-dimensional TROSY NMR spectroscopy and depend on the orientation angle of the molecules in the magnetic field, are variations along a

20 ^{15}N axis;

52. The program according to item 51, wherein the Saupe order matrix elements in (iii) are determined by:

with respect to the kth pair of ^{15}N nuclear

25 spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond having the kth pair of spins in the domain to the ith molecular axis,

providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth molecular axis,

setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N-H bond of 1.02 Å, or setting a static dipolar coupling D^0_{nh} at 21.7 kHz for a length of the N-H bond of 1.04 Å,

determining as δ_{ij} a tensor value for chemical shift anisotropy of a ^{15}N nucleus in an arbitrary residue of the protein, and

determining the Saupe order matrix elements S_{ij} defining the molecular orientation with respect to the magnetic field, by contacting the protein with the compound in the presence of the liquid crystalline material, providing $\Delta\delta$trosy(k) for the kth pair of ^{15}N nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the $\Delta\delta$trosy(k) together with the following equation (1):

$$\Delta\delta\text{trosy}(k) = \sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j + (2/3)\ \delta_{ij}\} \qquad (1)$$

i, j = x, y, z;

53. The program according to item 52, wherein in the step (a), the axial variations of NMR signals, which are generated by two-dimensional TROSY NMR spectroscopy and depend on the orientation angle of the molecules in the magnetic field, are variations along a ^{15}N axis;

54. The program according to item 53, wherein the Saupe order matrix elements in (vii) are determined

by:

with respect to the kth pair of ^{15}N nuclear spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond

5   having the kth pair of spins in the domain to the ith molecular axis,

providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth molecular axis,

10   setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N-H bond of 1.02 Å, or setting a static dipolar coupling D^0_{nh} at 21.7 kHz for a length of the N-H bond of 1.04 Å,

determining as δ_{ij} a tensor value for chemical

15   shift anisotropy of a ^{15}N nucleus in an arbitrary residue of the protein, and

determining the Saupe order matrix elements S_{ij} defining the molecular orientation with respect to the magnetic field, by making no contact of the protein

20   with the compound in the presence of the liquid crystalline material, providing $\Delta\delta$trosy(k) for the kth pair of ^{15}N nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the $\Delta\delta$trosy(k) together with the following equation (1):

25   $\Delta\delta$trosy(k) = $\sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j\ +(2/3)\ \delta_{ij}\}$ (1)

i, j = x, y, z;

55. The program according to item 52 or 54, wherein the comparison with respect to the information

on an orientation in the step (c) is carried out by:

(ix) providing directions of orientation
tensor axes by a first three unit vectors perpendicular
to each other, by using the information on an
orientation of the domain in the protein before the
protein is contacted with the compound, wherein the
first three unit vectors are expressed by

[0025]

$$\vec{e}_{fx}, \quad \vec{e}_{fy}, \quad \vec{e}_{fz}$$

(x) providing directions of orientation
tensor axes by a second three unit vectors
perpendicular to each other, by using the information
on an orientation of the domain in the protein after
the protein is contacted with the compound, wherein the
second three unit vectors are expressed by

[0026]

$$\vec{e}_{bx}, \quad \vec{e}_{by}, \quad \vec{e}_{bz}$$

(xi) denoting respective angles between the
individual first unit vectors and the individual second
unit vectors by a, b and c,

(xii) giving a degree of orientational change
by the following equation:

degree of orientational change $= a^2 + b^2 + c^2$

wherein the degree of orientational change is

compared;

56. The program according to item 48, further comprising means for identifying a position on the protein to which the compound is bound;

5   57. The program according to item 56, wherein the means of identifying a position on the protein to which the compound is bound is carried out by comparing the two-dimensional TROSY NMR spectrum obtained in the step (a) with the two-dimensional TROSY NMR spectrum

10 obtained in the step (b) to detect a spectral change, and identifying an amino acid residue in the protein which has induced the spectral change;

58. A program of selecting a second compound capable of inducing a structural change in a domain

15 within a protein on contact with the protein, which is similar to a structural change in the domain within the protein induced by a first compound on contact with the protein, comprising the means of:

(a) providing information on an orientation

20. of the domain when the protein is in contact with the first compound;

(b) providing information on an orientation of the domain when the protein is in contact with the second compound, by

25   (i) providing known atomic coordinates for the domain,

(ii) providing axial variations of NMR signals, which are generated from the protein in

contact with the second compound in the presence of a liquid crystalline material by two-dimensional TROSY NMR spectroscopy and depend on an orientation angle of the molecules in a magnetic field,

5   (iii) determining Saupe order matrix elements for the domain from the atomic coordinates for the domain and the axial variations of NMR signals, and

          (iv) diagonalizing the determined matrix to produce the information on an orientation of the

10   domain;

          (c) making a comparison between the information on an orientation provided in means (a) and the information on an orientation provided in means (b); and

15   (d) determining from the results obtained by the comparison in means (c) if the structural change in the protein induced by the second compound on contact with the protein is similar to the structural change in the protein induced by the first compound on contact

20   with the protein;

          59. The program according to item 25, wherein the means (a) is means of:

          (a) providing the information on an orientation of the domain when the protein is in

25   contact with the first compound, by

          (v) providing known atomic coordinates for the domain,

          (vi) providing axial variations of NMR

signals, which are generated from the protein in
contact with the first compound in the presence of the
liquid crystalline material by two-dimensional TROSY
NMR spectroscopy and depend on an orientation angle of
5 the molecules in the magnetic field,

(vii) determining Saupe order matrix elements
for the domain from the atomic coordinates for the
domain and the axial variations of NMR signals, and

(viii) diagonalizing the determined matrix to
10 produce the information on an orientation of the
domain;

60. The program according to item 58, wherein
the means (a) is means of

(a) providing the information on an
15 orientation of the domain from the atomic coordinates
provided previously when the protein was in contact
with the first compound;

61. The program according to item 58, wherein
in the step (b), the axial variations of NMR signals,
20 which are generated by two-dimensional TROSY NMR
spectroscopy and depend on the orientation angle of the
molecules in the magnetic field, are variations along a
^{15}N axis;

62. The program according to item 61, wherein
25 the Saupe order matrix elements in (iii) are determined
by:

with respect to the kth pair of ^{15}N nuclear
spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond having the kth pair of spins in the domain to the ith molecular axis,

providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth molecular axis,

setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N-H bond of 1.02 Å, or setting a static dipolar coupling D^0_{nh} at 21.7 kHz for a length of the N-H bond of 1.04 Å,

determining as δ_{ij} a tensor value for chemical shift anisotropy of a ^{15}N nucleus in an arbitrary residue of the protein, and

determining the Saupe order matrix elements S_{ij} defining the molecular orientation with respect to the magnetic field, by contacting the protein with the compound in the presence of the liquid crystalline material, providing $\Delta\delta$trosy(k) for the kth pair of ^{15}N nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the $\Delta\delta$trosy(k) together with the following equation (1):

$$\Delta\delta\text{trosy}(k) = \sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j +(2/3)\ \delta_{ij}\} \qquad (1)$$

i, j = x, y, z;

63. The program according to item 59, wherein in the step (a), the axial variations of NMR signals, which are generated by two-dimensional TROSY NMR spectroscopy and depend on the orientation angle of the molecules in the magnetic field, are variations along a

^{15}N axis;

64. The program according to item 63, wherein the Saupe order matrix elements in (vii) are determined by:

5   with respect to the kth pair of ^{15}N nuclear spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond having the kth pair of spins in the domain to the ith molecular axis,

10   providing ϕ^k_j as a vector angle of an N-H bond having the kth pair of spins in the domain to the jth molecular axis,

setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N-H bond of 1.02 Å, or setting

15 a static dipolar coupling D^0_{nh} at 21.7 kHz for a length of the N-H bond of 1.04 Å,

determining as δ_{ij} a tensor value for chemical shift anisotropy of a ^{15}N nucleus in an arbitrary residue of the protein, and

20   determining the Saupe order matrix elements S_{ij} defining the molecular orientation with respect to the magnetic field, by contacting the protein with the second compound in the presence of the liquid crystalline material, providing $\Delta\delta$trosy(k) for the kth

25 pair of ^{15}N nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the $\Delta\delta$trosy(k) together with the following equation (1):

$$\Delta\delta\text{trosy}(k) = \sum S_{ij}\{0.5 \ D^0_{nh} \cos\phi^k_i \ \cos\phi^k_j + (2/3) \ \delta_{ij}\} \quad (1)$$

i, j = x, y, z;

     65. The program according to item 62 or 64, wherein the comparison with respect to the information on an orientation in the step (c) is carried out by:

5   (ix) providing directions of orientation tensor axes by a first three unit vectors perpendicular to each other, by using the information on an orientation of the domain in the protein when the protein is contacted with the first compound, wherein

10   the first three unit vectors are expressed by

[0027]

$$\overrightarrow{e}_{ax}, \quad \overrightarrow{e}_{ay}, \quad \overrightarrow{e}_{az}$$

     (x) providing directions of orientation tensor axes by a second three unit vectors perpendicular to each other, by using the information

15   on an orientation of the domain in the protein when the protein is contacted with the second compound, wherein the second three unit vectors are expressed by

[0028]

$$\overrightarrow{e}_{bx}, \quad \overrightarrow{e}_{by}, \quad \overrightarrow{e}_{bz}$$

     (xi) denoting respective angles between the

20   individual first unit vectors and the individual second unit vectors by a, b and c and using the a, b and c as indices for the comparison with respect to the

information on an orientation;

66. The program according to item 62 or 64, wherein the comparison with respect to the information on an orientation in the step (c) is carried out by:

5   (ix) providing directions of orientation tensor axes by a first three unit vectors perpendicular to each other, by using the information on an orientation of the domain in the protein when the protein is contacted with the first compound, wherein

10   the first three unit vectors are expressed by [0029]

$$\overrightarrow{e}_{ax}, \overrightarrow{e}_{ay}, \overrightarrow{e}_{az}$$

(x) providing directions of orientation tensor axes by a second three unit vectors perpendicular to each other, by using the information

15   on an orientation of the domain in the protein when the protein is contacted with the second compound, wherein the second three unit vectors are expressed by [0030]

$$\overrightarrow{e}_{bx}, \overrightarrow{e}_{by}, \overrightarrow{e}_{bz}$$

(xi) denoting respective angles between the

20   individual first unit vectors and the individual second unit vectors by a, b and c, and

(xii) giving a degree of orientational

similarity to the active conformation by the following
equation:

degree of orientational similarity to the
active conformation = $a^2 + b^2 + c^2$

5   wherein the degree of orientational
similarity to the active conformation is used as index
for the comparison with respect to the information on
an orientation;

67. The program according to item 58, further
10   comprising the means of identifying a position on the
protein to which at least one of the first and second
compounds is bound;

68. The program according to item 67, wherein
the means of identifying a position on the protein to
15   which at least one of the first and second compounds is
bound is carried out by comparing a two-dimensional
TROSY NMR spectrum obtained in the absence of the
compound with a two-dimensional TROSY NMR spectrum
obtained in the presence of the compound to detect a
20   spectral change, and identifying an amino acid residue
which has induced the spectral change;
[0031]

69. An apparatus capable of carrying out the
method according to item 1, 13 or 25;
25   [0032]

70. An apparatus provided with the program
according to item 38, 48 or 58; and
[0033]

71. A storage medium containing the program according to item 38, 48 or 58.

ADVANTAGES OF THE INVENTION

[0034]

5   The present invention provides a method of estimating a conformational change in a target protein, which is induced by addition of a compound into a solution of the target protein, by a change thereof in a TROSY correlation NMR spectrum (for example, $^{1}H/^{15}N$

10   NMR spectrum) and a program therefor. The present invention provides a novel method capable of concurrent observation of 1) a site on a target protein to which a compound is bound, and 2) a structural change in the target protein induced by the compound.

15   BRIEF DESCRIPTION OF THE DRAWINGS

[0035]

        Fig. 1 illustrates a method of selectively identifying an agonistic lead compound based on a relative orientational change between domains;

20   Fig. 2 shows representations of orientation angles for a protein molecule;

        Fig. 3 is a photograph of a gel as alignment medium used in Example 3, where a white bar on the upper right shows a length of 10 mm;

25   Fig. 4 shows TROSY signals of an mRNA 5'-capping enzyme, which is the guanylyltransferase from

Chlorella virus PBCV1, in the gel as alignment medium;
the enzyme was analyzed in the stretched 6% (w/w) gel
of 50% acrylamide/50% acrylate (alignment medium) at
20°C (293K); and in the Figure., "I" represents data

5   acquired in an isotropic state, while "A" represents
data acquired in an aligned state;

Fig. 5 shows the results of analysis based on
an open crystal structure, where orientation tensors
were determined from 23 residues in the N-terminal

10 domain (large domain) and 28 residues in the C-terminal
domain (small domain);

Fig. 6 shows the results of analysis based on
an open crystal structure, where orientation tensors
were determined from 23 residues in the N-terminal

15 domain (large domain) and 28 residues in the C-terminal
domain (small domain);

Fig. 7 shows the results of analysis based on
a closed crystal structure, where orientation tensors
were determined from 21 residues in the N-terminal

20 domain (large domain) and 28 residues in the C-terminal
domain (small domain); and

Fig. 8 shows the results of analysis based on
a closed crystal structure, where orientation tensors
were determined from 21 residues in the N-terminal

25 domain (large domain) and 28 residues in the C-terminal
domain (small domain).

BEST MODE FOR CARRYING OUT THE INVENTION

[0036]

Hereinbelow the present invention is
described. It should be noted that all through this
specification, expression as a singular form includes
the concept of the plural referent unless otherwise
stated. Therefore, as for the articles for a singular
form (for example, "a", "an", "the", etc. in the case
of English), it should be understood that they include
concept of the plural referent unless otherwise stated.
In addition, it should be understood that all terms
used in this specification are used with a meaning
usually used in the art unless otherwise stated.
Accordingly, all technical and scientific terms used
herein have the same meaning as commonly understood by
those skilled in the art to which this invention
belongs unless defined otherwise. When they contradict
each other, this specification (including definitions)
takes priority.

[0037]

(Definition of terms)

The definitions of the terms used in this
specification in particular are listed below.

The terms "protein", "polypeptide",
"oligopeptide" and "peptide" used in this specification
are used in the same meaning in this specification, and
they refer to a polymer of amino acids of any length.
The polymer may be linear or branched or may be cyclic.
An amino acid may be natural or unnatural or may be a

modified amino acid. As for these terms, they can also be assembled into a complex of plural polypeptide chains. These terms also encompass a natural or artificially modified amino acid polymer. Such a

5 modification includes, for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation or any other manipulation or modification (for example, conjugation with a labeling component). This definition also encompasses, for

10 example, a polypeptide containing one or more analogs of amino acids (including, for example, unnatural amino acids), a peptide-like compound (for example, peptoid) and other modifications known in the art.

[0038]

15 A "domain" of a protein in this specification means a specific part of a protein molecule including one amino acid residue or at least two consecutive amino acid residues. The domain in this specification may have a specific function or no specific function on

20 its own.

[0039]

"An amino acid" may be natural or unnatural in this specification. A "derivative amino acid" or "amino acid analog" refers to one which is different

25 from the naturally occurring amino acids but has a function similar to the original amino acid. Such derivative amino acids and amino acid analogs are well-known in the art. The term "natural amino acids" mean

L-isomers of natural amino acids. Natural amino acids
are glycine, alanine, valine, leucine, isoleucine,
serine, methionine, threonine, phenylalanine, tyrosine,
triptophan, cysteine, proline, histidine, asparagic

5 acid, asparagine, glutamic acid, glutamine, γ-carboxy
glutamic acid, arginine, ornithine and lysine. All
amino acids as used in this specification are of L-type
unless indicated otherwise. The term "unnatural amino
acid" means an amino acid usually not found naturally

10 in proteins. Examples of unnatural amino acids include
norleucine, para-nitrophenylalanine, homophenylalanine,
para-fluorophenylalanine, 3-amino-2-benzylpropionic
acid, D- or L-type of homoarginine and D-phenylalanine.
An "amino acid analog" refers to a molecule which is

15 not an amino acid but similar to an amino acid in
physical properties and/or function. Examples of amino
acid analogs include ethionine, canavanine, 2-
methylglutamine. An amino acid mimic refers to a
compound which has a structure different from the

20 general chemical structure of an amino acid but
functions in a manner similar to a naturally occurring
amino acid.
[0040]

An amino acid can be mentioned in this

25 specification either by a publicly known three-letter
code thereof or by a one-letter code recommended by
IUPAC-IUB Biochemical Nomenclature Commission. A
nucleotide can be mentioned likewise by a commonly

accepted one-letter code.

[0041]

In this specification, an "equivalent" amino acid refers to an amino acid which has or is expected
5 to have an action similar to a certain amino acid in a protein or polypeptide molecule that is the basis of comparison, and particularly in an enzyme molecule, it refers to an amino acid which exists at a similar place in an active site and has a similar contribution to
10 catalytic activity.

[0042]

The term "receptor" used in this specification is a biological structure having one or more binding domains which complex with one or more
15 ligands reversibly and specifically, wherein this complex has a biological structure. A receptor may exist completely out of the cell (extracellular receptor), in the cell membrane (provided that parts of the receptor face the environment outside the cell and
20 the cytosol) or completely in the cell (intracellular receptor). They can also function independently of the cell. The receptor in the cell membrane enables the cell to communicate with the space outside the border (for example, signaling) and to function in the
25 transportation of a molecule or ion to the inside and to the outside of the cell. The receptor as used in this specification may be full length of the receptor or a fragment of the receptor.

[0043]

When a receptor fragment is used, for example, a site related to ligand recognition of receptor protein can be used. The site related to
5 ligand recognition of receptor protein can be identified as follows. The site related to ligand recognition can be estimated from the structure of proteins having high homology or similarity in function by homology and domain search. For example, when
10 calculation is performed with default parameters of BLAST on amino acid sequences of different receptor molecules which bind specifically to the same ligand, a region showing homology of 50% or more, preferably 55% or more, more preferably 60% or more, still more
15 preferably 65% or more is estimated as a ligand recognition site.

Furthermore, those skilled in the art can enable a gene encoding a variant receptor with deletion variation or amino acid substitution introduced to be
20 transiently expressed in an animal cell or the like, and easily determine the region which is essential to the function.

[0044]

The term "ligand" used in this specification
25 is a binding partner to a specific receptor or a family of receptors. The ligand may be an endogenous ligand to the receptor or alternatively it can be a synthetic ligand to the receptor such as a drug, drug candidate

or pharmacological means.

[0045]

An "agonist" in this specification refers to a factor which binds to a receptor for a certain

5 biologically active substance (ligand) and develops an effect identical to (or similar to) the effect of the substance.

[0046]

An "antagonist" in this specification refers

10 to a factor which competes against a certain biologically active substance (ligand) for binding itself to the receptor but develops no physiological effect of itself through the receptor. The antagonist also encompasses a reversal agent, a blocking agent

15 (blocker), an inhibitory agent (inhibitor), etc.

[0047]

"NMR" in this specification means magnetic nuclear resonance. When a population of nuclei having magnetic moments is placed in a static magnetic field

20 B_0, the nuclei are distributed at discontinuous energy levels according to the magnitude of the magnetic moment and the strength of the magnetic field by nuclear Zeeman effect. The phenomenon of resonance absorption observed when an electromagnetic radiation

25 having a frequency equivalent to a difference between these levels is exposed is referred to as magnetic nuclear resonance.

[0048]

"Two-dimensional NMR" in this specification refers to a method to develop a nuclear magnetic resonance spectrum in two frequency axes. Different pulses are used in this measurement and Fourier

5 transformation is performed using the time interval of the pulses and the time after the observation pulse as two time axes. Signal intensity is usually expressed by contour. Typically, two-dimensional NMR includes TROSY (transverse relaxation-optimized spectroscopy),

10 COSY(two dimensional shift correlation MNR method), SECSY(two dimensional spin echo correlation spectroscopy), FOCSY (two dimensional foldover-corrected spectroscopy), HOHAHA (two dimensional homonuclear Hartmann-Hahn spectroscopy), NOESY (two

15 dimensional NOE spectroscopy), 2D-J technique (two-dimensional J-resolved spectroscopy) and relayed COSY (relayed coherence transfer spectroscopy) but it is not limited to these. Preferable NMR is TROSY. TROSY is a method which has been developed by Wuthrich et al. and

20 suitable for super-high magnetic field NMR (Proc. Natl. Acad. Sci. USA vol. 94, pp. 12366-12371 (1997)). In NMR of a molecule having a high molecular weight, nuclear magnetic relaxation is governed by (1) magnetic dipolar interaction and (2) chemical shift anisotropy,

25 and the chemical shift anisotropy becomes very large in a super-high magnetic field, which shortens relaxation time and broadens line width. TROSY is a method which lengthens the relaxation time and makes narrower the

line width by offsetting these two interactions. TROSY
has enabled us to obtain a two-dimensional NMR spectrum
for a protein with a high molecular weight (for
example, 900 kDa or more).

5 [0049]

When two-dimensional NMR is performed in this
specification, nuclides of stable isotopes which are
used to label the protein include ^{15}N, ^{13}C, ^{2}H or a
combination thereof but they are not limited to these.

10 Preferable labelings using stable isotope nuclides
include ^{15}N single labeling, ^{13}C single labeling, $^{15}N/^{2}H$
double labeling, $^{13}C/^{2}H$ double labeling, and $^{15}N/^{13}C/^{2}H$
triple labeling.

[0050]

15 In this specification, "liquid crystal
material" is a material which causes a weak orientation
of the target protein. The liquid crystal material
includes a mixture of dimyristoylphosphatidylcholine
(DMPC) and dihexanoylphosphatidylcholine (DHPC),

20 a mixture of dimyristoylphosphatidylcholine
(DMPC) and dihexanoylphosphatidylcholine (DHPC) and
sodium dodecyl sulfate (SDS),

a mixture of dimyristoylphosphatidylcholine
(DMPC) and dihexanoylphosphatidylcholine (DHPC) and

25 cethyltrimethyl ammonium bromide (CTAB),

a mixture of 1,2-di-O-dodecyl-sn-glycero-3-
phosphocholine (DIODPC) and 3-(cholamidepropyl)-
dimethylammonio-2-hydroxy-1-propane sulfate (CHAPS),

a mixture of n-alkyl-poly (ethyleneglycol)/n-alkyl alcohol,

filamentous phage,

a mixture of cetyl pyridinium chloride

5 (CPCl)-hexanol-NaCl

a mixture of cetyl pyridinium bromide (CPBr)-hexanol-NaCl,

purple membrane fragments of Halobacterium genus,

10 microcrystalline cellulose and

polyacrylamide gel,

but it is not limited to these.

[0051]

When the liquid crystal material is a lipid,
15 bicelle made up of the lipid is mixed with the target protein, and the temperature of the mixture is set at phase transition temperature or more, the target protein can be weakly oriented.

[0052]

20 The "oriented state of molecule" in this specification is shown by Euler angle which shows tilt of coordinate axis of the molecule from the coordinate axis which assumes static magnetic field direction as Z-axis.

25 [0053]

The "information on an orientation" in this specification refers to information about orientation of domain in protein and refers to information obtained

as a molecular orientation tensor. For example, in this specification, information on an orientation can be expressed using unit vectors perpendicular to each other.

5 [0054]

In this specification, the "axial variations of NMR signals" refers to axial variations dependent on molecular orientation along the frequency axis of two-dimensional NMR developed for a stable isotope element.

10 For example, when ^1H is used, they refer to variations dependent on molecular orientation in the direction of ^1H axis and when ^{13}C is used, they refer to variations dependent on molecular orientation in the direction of ^{13}C axis.

15 [0055]

For a compound examined in the present invention, any compound can be used, and examples thereof include low molecular organic compounds, low molecular inorganic compounds, nucleic acids, nucleic

20 acid analogs, peptides, peptide analogs, metal ions, but it is not limited to these.

[0056]

When a low molecular organic compound or a low molecular inorganic compound is synthesized in the

25 present invention, combinatorial chemistry can be used. Combinatorial chemistry is a potent synthesizing process comprising introducing plural substituent groups into a certain basic scaffold by combinatorial

technique and synthesizing a certain group of molecules
(library) including a number of various compounds in a
short period of time and finding out a compound of
interest from this molecular group. Various kinds of

5   techniques for combinatorial chemistry are well-known.
Historically, the combinatorial chemistry is a
technique detonated by development of synthesis
technique based on "peptide synthesis" (1963) using
beads by Merrifield (U.S.A.) and "split synthetic

10 process" (1991) by Furka (Hungary) and the research and
development have been pushed forward energetically
since 1992. In particular, the solid phase synthesis
is a technique developing rapidly in late years since
it matches needs for speedup in that it does not

15 require purification after synthesis.
[0057]

        The terms "polynucleotide", "oligonucleotide"
and "nucleic acid" used in this specification are used
in the same meaning in this specification, and they

20 refer to a polymer of nucleotides of any length. These
terms also encompass a "derivatized oligonucleotide" or
"derivatized polynucleotide". The "derivatized
oligonucleotide" or "derivatized polynucleotide" refers
to an oligonucleotide or a polynucleotide which

25 contains a derivative of nucleotide or a binding
between nucleotides different from the ordinary one,
and they are used interchangeably. Specific examples
of such an oligonucleotide includes 2'-O-methyl-

ribonucleotide, derivatized oligonucleotides in which the phosphodiester bond in oligonucleotide is converted into a phosphorothioate bond, derivatized oligonucleotides in which the phosphodiester bond in

5 oligonucleotide is converted into an N3'-P5' phosphoroamidate bond, derivatized oligonucleotides in which the ribose and phosphodiester bond in oligonucleotide is converted into a peptide nucleic acid bond, derivatized oligonucleotides in which uracil

10 in oligonucleotide is replaced by C-5 propynyluracil, derivatized oligonucleotides in which uracil in oligonucleotide is replaced by C-5 thiazole uracil, derivatized oligonucleotides in which cytosine in oligonucleotide is replaced by C-5 propynylcytosine,

15 derivatized oligonucleotides in which cytosine in oligonucleotide is replaced by phenoxazine-modified cytosine, derivatized oligonucleotides in which ribose in DNA is replaced by 2'-O-propylribose and derivatized oligonucleotides in which ribose in oligonucleotide is

20 replaced by 2'-methoxyethoxy ribose. Unless otherwise indicated, a specific nucleic acid sequence is intended to also encompass the explicitly shown sequence as well as conservatively modified variants thereof (for example, degenerate codon substituted sequence) and the

25 complementary sequence. Specifically, the degenerate codon substituted sequence can be attained by making a sequence in which the third position of one or more selected (or all) codons are replaced by a mixed base

and/or a deoxyinosine residue (Batzer et al., Nucleic

Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol.

Chem. 260: 2605-2608 (1985); Rossolini et al., Mol.

Cell. Probes 8:91-98 (1994)). The term "nucleic acid"

5 is also used interchangeably with gene, cDNA, mRNA,

oligonucleotide and polynucleotide in this

specification. A specific nucleic acid sequence also

encompasses "splice variant". Similarly, a specific

protein encoded by a nucleic acid tacitly encompasses

10 any protein encoded by the splice variant of the

nucleic acid. A "splice variant" is a product of

genetic alternative splicing as the name suggests.

After transcription, the first nucleic acid transcript

can be spliced so that a different (or another) nucleic

15 acid splicing product encodes a different polypeptide.

Production mechanism of splice variant varies but

includes alternative splicing of exon. This definition

also encompasses another polypeptide derived from the

same nucleic acid by read-through transcription. This

20 definition encompasses any product of splicing reaction

(including a splicing product by recombination).

[0058]

"Nucleotide" may be natural or unnatural

thing in this specification. The "derivatized

25 nucleotide" or "nucleotide analog" refers to one

different from naturally occurring nucleotide but

having a function similar to the original nucleotide.

Such derivatized nucleotide and nucleotide analog are

well-known in the art. Examples of such derivatized
nucleotide and nucleotide analog include
phosphorothioate, phosphoramidate, methyl phosphonate,
chiral methyl phosphonate, 2-O-methyl ribonucleotide,

5   peptide-nucleic acid (PNA), but they are not limited to
these.
[0059]

In this specification, a "fragment" refers to
a polypeptide or polynucleotide having a sequence

10   length from 1 to n-1 when a full length polypeptide or
polynucleotide (length: n) is considered. The length
of a fragment can be suitably changed depending on the
object and, for example, and, the lower limit of the
length includes 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25,

15   30, 40, 50 or more amino acids in the case of
polypeptide, and, in addition, length expressed with an
integer not specifically listed here (for example, 11)
can be appropriate as the lower limit. In addition, in
the case of polynucleotide, nucleotides of 5, 6, 7, 8,

20   9, 10, 15, 20, 25, 30, 40, 50, 75, 100 and more are
included, and, in addition, length expressed with an
integer not specifically listed here (for example, 11)
can be appropriate as the lower limit. When used in
this specification, preferably a receptor "fragment"

25   specifically binds to the ligand to which the full
length receptor can specifically binds.
[0060]

The protein used in the present invention may

be purified from a natural source of supply or
recombinantly expressed by transforming a host cell by
means of genetic engineering. The method of
genetically engineering a polypeptide includes, for

5   example, a method comprising culturing bacteria which
are prokaryote producing the polypeptide and having the
recombinant receptor protein accumulated as inclusion
body in the bacteria and obtaining the polypeptide by
disrupting the host bacteria.

10   [0061]

The "transformant" refers to the whole or
part of an organism such as a cell created by
transforming a host cell. As a transformant, a
prokaryotic cell is exemplified. A transformant is

15   also referred to as a transformed cell, transformed
tissue, transformed host or the like depending on the
target and all of these forms are included in this
specification, but it can indicate a specific form in a
specific context.

20   [0062]

Host bacteria cells to obtain a transformant
are not particularly limited as long as they express a
polypeptide which maintains bioactivity, and various
host bacteria cells conventionally used in gene

25   manipulation can be used. As prokaryotic cells,
prokaryotic cells belonging to genus Escherichia, genus
Serratia, genus Bacillus, genus Brevibacterium, genus
Corynebacterium, genus Microbacterium, genus

Pseudomonas and the like, for example, Escherichia coli

XL1-Blue, Escherichia coli XL2-Blue, Escherichia coli

DH1, Escherichia coli MC1000, Escherichia coli KY3276,

Escherichia coli W1485, Escherichia coli JM109,

5 Escherichia coli HB101, Escherichia coli No, 49,

Escherichia coli W3110, Escherichia coli NY49,

Escherichia coli BL21(DE3), Escherichia coli

BL21(DE3)pLysS, Escherichia coli HMS174(DE3),

Escherichia coli HMS174(DE3)pLysS, Serratia ficaria,

10 Serratia fonticola, Serratia liquefaciens, Serratia

marcescens, Bacillus subtilis, Bacillus

amyloliquefaciens, Brevibacterium ammmoniagenes,

Brevibacterium immariophilum ATCC14068, Brevibacterium

saccharolyticum ATCC14066, Corynebacterium glutamicum

15 ATCC13032, Corynebacterium glutamicum ATCC14067,

Corynebacterium glutamicum ATCC13869, Corynebacterium

acetoacidophilum ATCC13870, Microbacterium

ammoniaphilum ATCC15354, Pseudomonas sp. D-0110 can be

exemplified.

20 [0063]

As for the polypeptide derived from cells

obtained according to the present invention, one or

more amino acids in the amino acid sequence may be

replaced, added and/or deleted and a sugar chain may be

25 replaced, added and/or deleted as long as it has

substantially the same effect as a polypeptide of

natural type.

[0064]

A certain amino acid can be replaced by another amino acid in a protein structure for example, on a binding site of a ligand molecule, without apparently decreasing or losing its interactive binding

5 ability. It is the interactive binding ability and property of a protein that determine the biological function of the protein. Therefore, replacement of a specific amino acid in a protein can be performed in an amino acid sequence or at a level of a coding DNA

10 sequence, with the protein still maintaining the original property can be created after the replacement. Therefore, various kinds of modifications can be performed in a peptide or a corresponding DNA encoding this peptide disclosed in this specification without

15 clear loss of biological utility.

[0065]

When designing modification as mentioned above, hydrophobicity index of amino acids can be taken into consideration. Significance of hydrophobicity

20 index of amino acids for giving interactive biological function in a protein is generally recognized in the art (Kyte J. and Doolittle, R. F., J. Mol. Biol. 157 (1): 105-132, 1982). Hydrophobicity of an amino acid contributes to the secondary structure of the generated

25 protein and subsequently determines the interaction of the protein with other molecules (for example, enzyme, substrate, receptor, DNA, antibody, antigen, etc.). Each amino acid is assigned with a hydrophobicity index

based on the hydrophobicity and a property of charge.
They are: isoleucine (+4.5); valine (+4.2); leucine
(+3.8); phenylalanine (+2.8); cysteine/cysteine (+2.5);
methionine (+1.9); alanine (+1.8); glycine (-0.4);

5   threonine (-0.7); serine (-0.8); triptophan (-0.9);
tyrosine (-1.3); proline (-1.6); histidine (-3.2);
glutamic acid (-3.5); glutamine (-3.5); asparagic acid
(-3.5); asparagine (-3.5); lysine (-3.9); and arginine
(-4.5).

10 [0066]

It is well-known in the art that another
protein still having a similar biological function (for
example, a protein which is equivalent in ligand
binding capacity) can be produced by replacing a

15 certain amino acid with another amino acid having a
similar hydrophobicity index. The hydrophobicity index
is preferably within ±2, more preferably within ±1, and
still more preferably within ±0.5 in such amino acid
replacement. It is understood in the art that such

20 replacement of amino acid based on hydrophobicity is
effective. As described in US Patent No. 4,554,101,
the following hydrophilicity indexes are assigned to
amino acid residues: arginine (+3.0); lysine (+3.0);
asparagic acid (+3.0±1); glutamic acid (+3.0±1); serine

25 (+0.3); asparagine (+0.2); glutamine (+0.2); glycine
(0); threonine (-0.4); proline (-0.5±1); alanine (-
0.5); histidine (-0.5); cysteine (-1.0); methionine (-
1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8);

tyrosine (-2.3); phenylalanine (-2.5); and triptophan

(-3.4). It is understood that an amino acid can be

replaced with another amino acid having a similar

hydrophilicity index and still giving a biological

5   equivalent. The hydrophilicity index is preferably

within ±2, more preferably within ±1, and still more

preferably within ±0.5 in such an amino acid

replacement.

[0067]

10   In the present invention, the "conservative

replacement" refers to a replacement in which the

hydrophilicity index and/or hydrophobicity index

resembles as above in the amino acid replacement

between the original amino acid and the replaced amino

15   acid. Examples of conservative replacement are well-

known to those skilled in the art and, for example, it

is replacement in each of the following groups:

arginine and lysine; glutamic acid and asparagic acid;

serine and threonine; glutamine and asparagine; and

20   valine, leucine and isoleucine but it is not limited to

these.

[0068]

        In this specification, the "variant" refers

to one partly changed for the original substance such

25   as polypeptide or polynucleotide. Such a variant

includes replacement variant, addition variant,

deletion variant, trucated variant, allele variant,

etc. Alleles refer to genetic variants which belong to

the same locus but can be distinguished from each other. Therefore, the "allele variant" refers to a variant having a relation of an allele to a certain gene. A "species homolog or homolog" refers to one

5 which has homology (preferably it is homologous by 60% or more, more preferably it is homologous by 80% or more, 85% or more, 90% or more, or 95% or more) with a certain gene in a species at an amino acid level or a nucleotide level. The method for acquiring such a

10 species homolog is clear from the description of this specification. An "ortholog" is also referred to as orthologous gene and, refers to a gene where two genes derive from the common ancestor through speciation. For example, when hemoglobin gene family having

15 multigene structure is taken as an example, human and mouse α hemoglobin genes are orthologs while human α hemoglobin gene and β hemoglobin gene are paralogs (genes resulted from gene redundancy). Orthologs are useful for estimation of molecule dendrogram and

20 therefore, orthologs can also be useful in the present invention.

[0069]

A "conservative (modified) variant" is applied to both amino acid sequence and nucleic acid

25 sequence. With regard to a specific nucleic acid sequence, a conservatively modified variant refers to a nucleic acid encoding the same or essentially the same amino acid sequence and when the nucleic acid encodes

no amino acid sequence, it refers to essentially the same sequence. Due to degeneracy of genetic code, a plurality of functionally identical nucleic acids encode an arbitrarily appointed protein. For example,

5 all codons GCA, GCC, GCG and GCU encode an amino acid alanine. Therefore, at all positions where alanine is specified by a codon, the codon can be changed to any one of the described corresponding codons without changing the encoded polypeptide. Such a change in

10 nucleic acid is a "silent modification (variation)", one of the conservatively modified variant. All nucleic acid sequences in this specification encoding a polypeptide also describe all possible silent variations of the nucleic acid. It is understood in

15 the art that each codon in nucleic acids (except AUG which is usually the only codon for methionine and TGG which is usually the only codon for tryptophan) can be changed so as to produce functionally the same molecule. Therefore, each silent variation of nucleic

20 acid encoding a polypeptide is tacitly included in each of the described sequence. Preferably such a change can evade replacement of cysteine, an amino acid which has a great influence on higher-order structure of a polypeptide.

25 [0070]

In addition to replacement of an amino acid, addition, deletion or modification of an amino acid can be performed so as to make a functionally equivalent

polypeptide in this specification. Replacement of an amino acid refers to replacement in which one or more, for example, 1 to 10, preferably 1 to 5, more preferably 1 to 3 amino acids are replaced in the original peptide. Addition of an amino acid refers to addition in which one or more, for example, 1 to 10, preferably 1 to 5, more preferably 1 to 3 amino acids are added to the original peptide. Deletion of an amino acid refers to deletion in which one or more, for example, 1 to 10, preferably 1 to 5, more preferably 1 to 3 amino acids are deleted from the original peptide. Amino acid modification includes amidation, carboxylation, sulfation, halogenation, alkylation, glycosylation, phosphorylation, hydration, acylation (for example, acetylation), but it is not limited to these. A replaced or added amino acid may be a natural amino acid or may be an unnatural amino acid or an amino acid analog. A natural amino acid is preferable. [0071]

Such a nucleic acid can be obtained by well-known PCR method and can be also synthesized chemically. These methods may be combined, for example, with site-directed mutagenesis, hybridization method, etc.

[0072]

In this specification "replacement, addition or deletion" of a polypeptide or polynucleotide refers to replacement, addition or deletion in which an amino

acid or a substitute thereof or a nucleotide or a
substitute thereof is replaced by, added to or deleted
from the original polypeptide or polynucleotide,
respectively. Techniques for such replacement,

5   addition or deletion are well-known in the art and
examples of such techniques include site-directed
mutagenesis technique. Replacement, addition or
deletion may be performed in any number not less than
one and such a number can be increased as far as the

10   function to be aimed in variant having the replacement,
addition or deletion (for example, cancer marker, nerve
disease marker) is maintained. For example, such a
number can be 1 or several and preferably it can be 20%
or less, or 10% or less of the total length, or 100 or

15   less, 50 or less, 25 or less, or the like.
[0073]

        Polymer structures (for example, polypeptide
structure) can be described for construction at various
levels. As for general discussion on this

20   construction, see for example, Alberts et al.,
Molecular Biology of the Cell (third edition, 1994) and
Cantor and Schimmel, Biophysical Chemistry Part I: The
Conformation of Biological Macromolecules (1980). The
"primary structure" refers to a specific amino acid

25   sequence of peptides. The "secondary structure" refers
to a three-dimensional structure locally positioned in
a polypeptide. Generally these structures are well
known as domains. A domain is a part of a polypeptide

which forms a minute unit of a polypeptide and typically it has a length of 50 to 350 amino acids. Representative domains are made of parts such as β sheet (β strand) and stretch of α-helix. The "tertiary

5   structure" refers to a complete three-dimensional structure of polypeptide monomers. The "quaternary structure" refers to a three-dimensional structure formed by a noncovalent association of independent tertiary units. Terms on anisotropy are used in the

10 same way as the terms known in the field of energy.
    [0074]

         The term "biomolecule" used in this specification refers to a molecule related to a living body. The "living body" in this specification refers

15 to a biological organism, and includes animal, plant, fungi, virus, but it is not limited to them. The biomolecule encompasses molecules extracted from the living body, but they are not limited to these and any molecules which can affect the living body are included

20 in the definition of biomolecule. Such a biomolecule includes protein, polypeptide, oligopeptide, peptide, polynucleotide, oligonucleotide, nucleotide, nucleic acid (including, for example, DNA such as cDNA and genome DNA, RNA such as mRNA), polysaccharide,

25 oligosaccaride, lipid, low molecular compound (for example, hormone, ligand, information transmitter, organic low molecular compound, combinatorial library compound), complex molecule thereof, but it is not

limited to these. The biomolecule which is preferable in this specification is a receptor, a receptor fragment and a ligand thereof.

[0075]

5   When a receptor is used as a protein in this specification, the receptor includes scavenger receptor including lectin-like oxidation LDL receptor (LOX-1), receptor belonging to insulin receptor family, receptor belonging to EGF receptor family, receptor belonging to

10   PDGF receptor family, receptor belonging to VEGF receptor family, receptor belonging to FGF receptor family, growth factor receptor such as NGF receptor family and TGF-β super family receptor, receptor of Toll-like receptor family, receptor of LDL receptor

15   association protein family and receptor of G protein conjugate type receptor family, but it is not limited to these.

[0076]

The present invention provides a technique to

20   read a conformational change in a target protein, which is induced by addition of a compound into a solution of the target protein, from a change in TROSY NMR correlation spectrum (for example, ^{1}H/^{15}N-NMR spectrum). By this technique, both of 1) a site on a target

25   protein to which a compound is bound and 2) a structural change in the target protein caused by the compound can be observed at the same time.

[0077]

Most of proteins develop their function when they are converted into an active structure by a compound such as ligand and agonist. Therefore, a compound which converts the target protein to an active structure can be an agonistic lead compound, and on the other hand, a substance which inhibits the conversion of the target protein to an active structure by ligand, agonist, etc. can be a lead compound for screening a novel antagonist. Therefore, it is important in the screening of new drugs to detect how the structure of the protein changes when a compound is bound or contacted with the target protein.

[0078]

However, technique of analyzing interaction between a compound and the target protein by existing NMR does not provide quantitative data relating to the change in protein structure. Therefore, a method to detect how the structure of the protein changes when a compound is bound or contacted with the target protein is desired.

[0079]

The present invention provides a method of determining a structural change of the protein when a compound is bound to the protein by using magnetic nuclear resonance and a method therefor and a program used in the method.

[0080]

More specifically, the present invention

relates to a method of determining a structural change of the protein when an arbitrary compound is contacted with and bound to with the protein by using magnetic nuclear resonance. Furthermore, the present invention

5 provides a method of identifying and screening a compound causing a structural change of the protein when an arbitrary compound is bound or contacted with the protein. In addition, the present invention provides a method of identifying and screening a

10 substance which induces a structural change similar to that induced by a ligand or an agonist, by comparing a structural change when a substance such as, for example, a ligand or an agonist, which is known to induce a structural change in a protein when bound to

15 the protein, is bound to the protein and a structural change when an arbitrary compound is bound to the protein. For example, the present invention provides quantitative information of a change in the protein structure caused by a compound and therefore a

20 screening method which enables identification of an agonistic lead compound.

[0081]

This method is based on the use of anisotropic spin interaction which is observed by

25 allowing proteins weakly oriented in NMR magnetic field. A number of researches have been reported about acquisition of information on an orientation of each binding axis in the protein from the static magnetic

field, which is caused by residual dipole effect, one of the anisotropic spin interaction, and application thereof to the analysis of the protein structure. In addition, technique to decide relative orientation between domains constituting a protein based on the residual dipole effect has been also reported. Observation of the residual dipole effect imposes, however, a theoretical limitation on the molecular weight of the target protein which can be observed and only proteins of about 60 kDa (about 500 residues in the number of amino acid) or less may be observed. Target proteins suitable to screen drugs have a molecular weight much larger than 60 kDa. Therefore, it has been too inadequate in versatility to be applied for screening compounds aimed at drug discovery.

[0082]

The new technique which is a key in the present invention enables to exceed the application limit of the molecular weight of the target protein which resides in the conventional method and to quantitatively observe relative orientation between domains in a protein caused by a compound even when a protein of 900 kDa or more is directed to theoretically and to determine the a structural change.

[0083]

For example, the present invention provides a screening technique to search for a lead compound for a novel drug. Specifically, the present invention

provides a technique to quantitatively evaluate a structural change of a target protein derived from interaction with a compound by TROSY $^1H/^{15}N$ correlation NMR spectrum or TROSY $^2H/^{15}N$ correlation NMR spectrum.

5   Furthermore, the present invention makes use of this technique and provides a screening method for identifying agonistic lead compound using as an index the presence or absence of the change of the target protein to an active structure.

10   [0084]

(NMR structure analysis)

        As for the protein sample used for higher-order structure analysis by NMR used in this specification, it is usually subjected to structure

15   analysis by labeling with a stable isotope through biosynthesis. Generally, an expression system for the target protein is established at first by genetically manipulating E. coli, etc., which is then expressed in a culture medium to which carbon sources and nitrogen

20   sources labeled with stable isotopes (glucose in which all carbons are labeled with ^{13}C and ammonium chloride in which nitrogens are labeled with ^{15}N) are added, and thereby proteins with all the carbons and nitrogens labeled with stable isotopes can be obtained. The

25   resulting labeled proteins can be purified by chromatography or the like, concentrated by ultrafiltration and subjected to NMR measurement. On this occasion, it is desirable to pay attention to the

following conditions.

[0085]

As for container to be used, apparatuses to be used including a NMR sample tube is preferably subjected to coating with silicone, etc. to suppress adhesion and spreading of the protein on the glass wall surface in consideration of high viscosity of the protein. It is also desirable to use Pasteur pipettes in handling a sample.

[0086]

As a solvent, an aqueous solution is usually used. A protein has, however, a lot of exchangeable amide protons and when a heavy water solution is used, amide protons, which are a key factor in the analysis of spectrum, are substituted with deuterium resulting in loss of a lot of information. Therefore, usually a sample is prepared with a light water solution, and then heavy water of on the order of 10% is added for NMR locking. A surfactant, a reducing agent may be added as required.

[0087]

Sample concentration is usually on the order of 1 mM (for example, 0.4 mM to 2 mM). If the sample is excessively available and solubility is high, measurement can be performed even by 5 mM to 10 mM but it should be noted that association in the solution could occur at a higher concentration. In order to prevent unexpected analysis results resulted by

association, it is preferable to compare linearity using samples of a lower concentration and a higher concentration, and to confirm whether association occurs or not.

5 [0088]

pH to be used is usually preferably around 5 to 7. For exchanging rate of exchangeable proton such as amide proton is decreased by lowering pH. However, low pH may affect the higher-order structure and

10 therefore, it is preferable to confirm by circular dichroism (CD) spectrum, etc.

[0089]

As for buffer, those having no protons are preferably used to prevent them from interfering the

15 spectral analysis. Such a buffer includes phosphate buffer, deuteration acetate buffer but it is not limited to these.

[0090]

The temperature to be used is desirably

20 around 30 to 40°C to get closer to the temperature in the living body. Because T2 of protein molecule gets longer by setting the temperature rather high and lowering the viscosity of the solution, spectrum is generally obtained in a good condition when the

25 temperature is high.

[0091]

When dissolved oxygen is contained in the sample solution, relaxation time shortens due to

paramagnetic relaxation which causes loss in the
sensibility and may be an obstacle to detect the TROSY
correlation and therefore, it is preferable to remove
dissolved oxygen by deaeration. Deaeration operation

5 includes deaeration under reduced pressure, bubbling of
inert gas, and preferably vacuum deaeration is used.
It is desirable not to make foams.
[0092]

For the conservation of solution, it is

10 desirable to add azide, but it is not always necessary
to add it.
[0093]

After a sample is prepared, shimming of NMR
is performed. At first NMR locking is made valid and

15 resolution adjustment is performed. Since NMR
measurement of a protein solution is observation in a
light water solution as a general rule, it is desirable
to cancel the huge water signal (with a concentration
of ^{1}H higher than the target protein by tens of

20 thousands to millions times). This is performed for
the purpose of adjusting resolution, and preferably it
is desirable to perform sufficient resolution
adjustment for every measured sample in order to obtain
high quality spectrum. Usually, the sample tube is not

25 rotated in the measurement of protein solution NMR, it
is necessary to surely increase the resolution not only
for Z-axis but also for X- and Y-axes as well as
adjustment of high ordered term in order to obtain a

good spectrum.

[0094]

Next, pulse width is measured. It is necessary to measure the pulse width for every measured sample in principle since the pulse width may vary for samples according to the difference of salt intensity in the protein solution NMR measured in an aqueous solution.

[0095]

Since the pulse sequence used in protein solution NMR has a number of RF pulses arranged, it is assumed that shift of pulse width as a total results in considerable influences. Therefore, it is desirable that before carrying out multiple dimension NMR measurement, pulse width is measured with a signal of the target protein and the multiple dimension NMR measurement is performed using the value.

[0096]

Then the sample is checked by NMR. It is desirable to perform checking by NMR in such a case where the protein may have deteriorated after stored for a certain period of time from the sample preparation.

[0097]

That is, usually $^{15}N-^{1}H$ HSQC measurement is performed and checking is performed by two-dimensional NMR spectrum. When there are inadequacies found such as inappropriate signal pattern or unmatched phase,

adjustment of experimental conditions is again
necessary because target protein may be degenerated.
[0098]

5   Next, performed is spectral analysis
(assignment of signals). Outline of analytical method
of spectrum is described below taking NOE spectrum as
an example. ^{1}H signals are assigned based on the
distance information derived from NOE obtained by NMR
and a higher-order structure can be derived. All of
10 the signals overlapped several folds are separated by
using 3 dimension measurement, 4 dimension measurement
and like and assigned to the known amino acid sequence.
The principle is to use the facts that spectrum has a
specific pattern for every amino acid and there are
15 extremely characteristic rules in the chemical shifts
and spin coupling constants. Automatic assignment is
also performed now utilizing such characteristic
properties. Characteristic chemical shifts of protein
NMR spectrum are shown below.
20 [0099]

     Chemical shifts of ^{1}H in a protein are
generally specified by every environment. In other
words, a signal derived from an amide proton (in the
neighborhood of 8 ppm), signals derived from an
25 aromatic ring at a side-chain (in the neighborhood of 7
ppm), signals derived from α-position protons (in the
neighborhood of 4 ppm), signals derived from β-position
protons (in the neighborhood of 2 to 3 ppm) and signals

derived from a methyl group (in the neighborhood of 1 ppm) are respectively observed.

[0100]

There are similar correlations in the chemical shift of ^{13}C, and in the case of ^{13}C, it is utilized in various measuring method for assignment by selectively activating each area and handling carbons of α-position and β-position, carbonyl carbon as if they were other nuclides.

[0101]

As well as assignment of amino acid sequence, a change in the chemical shift derived from higher-order structure is observed. For example, signal derived from a proton at α-position shifts to higher magnetic field in an α-helix structure and shifts to lower magnetic field in β-sheet structure. In addition, there may be a shift around ±1 ppm depending on the positional relationship with an aromatic ring of the side-chain. Further, considerably large shift is observed in metalloprotein at a part where it binds a metal.

[0102]

Next, spin coupling between the same nuclides should be taken into consideration. Since amino acids constituting protein are linked by a peptide bond (amide bond), spin coupling between $^1H-^1H$ is divided by a carbonyl group, and the spin coupling between $^1H-^1H$ is limited within the same amino acid residue. Therefore,

pattern of spin coupling between ^{1}H–^{1}H is characteristic

of each kind of amino acid and amino acids can be

identified using this fact. In addition, dihedral

angle ϕ derived from ^{3}JH$_\alpha$H$_N$ spin coupling constant

5 between α-position proton and amide proton helps to

determine the secondary structure of the main chain.

[0103]

Nuclear spin coupling between different

nuclides should also be taken into consideration.

10 Examples of spin coupling constant except ^{1}H–^{1}H related

to main chain include H–N(90–100 Hz), N–Cα (11 Hz), Cα–

H(140 Hz), Cα–Cβ(30–40 Hz), Cα–C(=O)(55 Hz), Cα–X–N(7

Hz), ^{13}C–^{15}N(15 Hz).

[0104]

15 In addition, NOE correlation should also be

taken into consideration. NOE correlation is used for

higher-order structure analysis, and ^{1}H same nuclide NOE

sandwiching a carbonyl group with amide proton and α-

position proton shows that the two protons come from an

20 amino acid residue adjacent to each other through an

amide bond, and is used by assignment of amino acid

sequence by ^{1}H same nuclide experiment. This technique

together with classification information of the amino

acid determined based on spin coupling and chemical

25 shift refers to sequence specific chain assignment

method.

[0105]

When assignment procedure is over, higher-

order structure analysis is performed if necessary. The higher-order structure of protein includes secondary structure such as α-helix structure and β-sheet structure which amino acid sequence forms with a

5 steric regularity, tertiary structure which some secondary structures are disposed spatially, quaternary structure which domains constructed by tertiary structures are disposed spatially, and it is important to clarify these structures in structure analysis in

10 protein solution NMR measurement, which is performed in various ways according to the purpose.

[0106]

When the primary sequence is a known amino acid sequence, the way how the known polypeptide chains

15 are entangled with each other to construct a protein is clarified as an image using information provided by NMR. What is used on this occasion is structure optimization calculation algorithm called distance geometry method, molecular dynamics method with binding

20 condition. In either case, these are techniques in which NOE signals as distance information obtained from NMR are gathered as much as possible, which are then given to the calculation program as parameters to work out higher-order structure.

25 [0107]

In this specification, the "distance geometry method" refers to a technique which uses a set of distance information and bond angle (dihedral angle) as

spatial positional information and the structure is derived based on these. Usually, bond length and bond angle of the covalent bond are fixed at standard values of common proteins, and structural calculation is

5   conducted using the dihedral angle as a variable. Some of the structures which have converged in such a direction as satisfying the binding condition of distance derived from NOE are extracted, and the structure that seems most appropriate is assumed as the

10   final structure. Because this technique involves a little number of variables, calculation is fast, and load on a computer is light, but change of the structure is limited by the contact of atoms (passing between covalent bonds is not possible) and therefore

15   it is difficult to converge into the true structure, and it is used to give the initial structure such as used in molecular dynamics method with binding condition described below.

[0108]

20   In this specification, the "molecular dynamics method with binding condition" is one of the molecular dynamics method (method of virtually oscillating molecules to optimize the structure) known as a technique which is generally used to simulate

25   molecular motion and refers to a technique in which the binding condition of distance derived from NOE is added as potential to optimize the structure. Since this technique uses Cartesian coordinates as positional

information and involves a number of variables,
enormous amount of calculation is needed and load on a
computer is heavy, but it is often used at present
because calculation time is shortened by rapid progress
5 of computing power of computer.

[0109]

In this specification, the "simulated
annealing method" is one of the techniques of molecular
dynamics calculation and refers to a technique in
10 which, unlike common molecular dynamics calculation,
parameters contributing to covalent bond are set weak
at an initial stage of calculation, temperature of the
system is rapidly elevated to aggravate the molecular
motion and then the temperature of the system is
15 gradually lowered to slow down the molecular motion as
well as to strengthen respective parameter, and thereby
converging the structure. According to this, apparent
convergence of structure resulted by inhibition of
change of structure due to contact of atoms can be
20 prevented.

[0110]

When the 3-dimension or 4-dimension developed
NOE spectrum as mentioned above is observed, detection
of correlation correlation signals between different
25 nuclide spins, i.e. $^1H-^{15}N$ or $^1H-^{18}C$ is basic. When a
high molecular weight protein (30 kDa or more) is an
object to be analyzed, it becomes difficult to detect
these correlation signals between different nuclides

due to rapid spin relaxation resulted from shortened T2 relaxation time of each nuclear spin. As a method to solve the problem of rapid spin relaxation which cannot be avoided when a high molecular weight protein is to

5 be observed, and to enable observation of a correlation signal of different nuclides with sufficient observation sensibility even for high molecular weight proteins, TROSY was developed by the group of Wuthrich. This new detection technique of correlation signals

10 between different nuclides can be incorporated into an existing measuring method which has been used for determining conformation of protein till now and the protein structure analysis process mentioned above can be applied to a protein having a molecular weight of 30

15 kDa or more by changing the data measuring method to a method using TROSY.

[0111]

Hitherto, NMR techniques usable in this specification are described, but such techniques are

20 well known in the art, and described, for example, Kurt Wuthrich, translated by Yoshimasa Kyougoku/Yuji Kobayashi, NMR of protein and nucleic acid: Structure analysis by two-dimensional NMR, Tokyo Kagaku Dojin, (1991); M.Sattler, J.Schleucher, C.Griesinger, Progr.

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5   Nemoto, Takuya Yoshida, Yuji Kobayashi, Kagaku-to-Kogyo

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Nobuaki Nemoto, Takuya Yoshida, Yuji Kobayashi, Kagaku-

to-Kogyo (Science and Industry), 70 70 (2), 48-55.

(1996), which are entirely incorporated in this

10 specification by reference.

[0112]

(Identification of lead compound of agonist)

    One embodiment of identifying a lead compound

of agonist is described below. A protein is formed by

15 connecting domains which are partial structures shown

as ellipses in Fig. 1. Many proteins, when developing

the function, change the relative configuration between

these domains into the structure most suitable for

developing the function. The induction to the active

20 structure is usually performed by binding of a low

molecular weight compound to the protein. An inhibitor

binds to the protein but does not induce it to an

active structure and functions to maintain the inactive

structure. On the other hand, in the case of a

25 compound activating the protein, it binds to the

protein and thereby induces a conformational change

most suitable for developing the function. Whether the

protein structure induced by the binding of a compound

is an active structure or not can be judged by using as a template a conformation induced when bound to a natural substrate. The present invention enables to judge whether the protein is in an active structure or

5   not from an easily observable experimental value, i.e. TROSY spectral change by providing NMR analysis technique to quantitatively determine a relative orientation between domains of protein. By using this technique, a lead compound of agonist can be

10   selectively identified based on a relative orientation change between the domains depicted in Fig. 1 as an image.

[0113]

(Calculation method of molecular orientation of

15   protein)

Calculation necessary for determining the molecular orientation of protein using an NMR spectrum can be performed, for example as follows, but it is not limited to this.

20   [0114]

$\Delta\delta$ trosy(k), molecular orientation dependent variation of a signal observed on TROSY spectrum in the direction along the ^{15}N axis can be linked with the two values: residual dipole effect between ^{1}H-^{15}N nuclear

25   spins Dnh and molecular orientation dependent variation of a signal in the direction along the ^{15}N axis resulted from spatial anisotropy of electron distribution around ^{15}N nucleus by the following relationship:

[0115]

$$\Delta\delta\text{trosy} = (1/2)D_{nh} + \Delta\delta^{15}N \qquad (1)$$

The residual dipole effect D_{nh} can be expressed with an angle ϕ_i, angle of each NH bond spectrum in the main chain of a protein against the i-th molecule coordinate axis (i = x, y, z) and Saupe order matrix, S defining the orientation of molecules against the external magnetic field (S_{ij} represents an element of molecular orientation matrix; i,j = x, y, z) by the following relationship. D^0_{nh} represents intensity of a dipolar interaction between ^1H–^{15}N.

[0116]

$$D^k_{nh} = D^0_{nh}\Sigma \; S_{ij} \cos \phi^k_i \cos \phi^k_j \qquad (2)$$
$$i,j = x,y,z$$

On the other hand, $\Delta\delta^{15}N$, a change in the chemical shift of ^{15}N derived from the orientation dependent difference of magnetostatic shielding against external magnetic field of ^{15}N nucleus against external magnetic field, which is resulted from anisotropy of electron distribution around ^{15}N nucleus, can be expressed with σ_{ij} (i,j = x,y,z), elements of chemical shift anisotropy tensor inherent to each peptide bond expressed on molecule coordinate axis and Saupe order matrix defining molecular orientation by the next formula:

[0117]

$$\Delta\delta^{15}N = (2/3) \; \Sigma \; S_{ij}\delta_{ij} \qquad (3)$$
$$i,j = x,y,z$$

From the relation of formulae (1) to (3), $\Delta\delta$trosy, variation of TROSY signal in the direction along the ^{15}N axis dependent on orientation angle of molecule in the magnetic field, can be expressed by the

5   next formula:

[0118]

$$\Delta\delta\text{trosy }(k) = \Sigma\, S_{ij}\, \{(1/2)\, D^0_{nh}\, \cos\, \phi^k_i\, \cos\, \phi^k_j +$$
$$(2/3)\, \delta_{ij}\} \tag{4}$$
$$i,j = x,y,z$$

10   Using the relationship of formula (4) obtained for plural signals k observed on spectrum and by Singular Value Decomposition, every element S_{ij} of the Saupe order matrix that it is necessary to decide molecular orientation can be determined from

15   experimental value $\Delta\delta$trosy and the value $\{(1/2)\, D^0_{nh}\, \cos\, \phi_i\, \cos\, \phi_j + (2/3)\, \delta_{ij}\}$ calculated from atomic coordinates within domain structure of the target protein. In this case, the relationship of formula (4) obtained for at least 5, preferably at least 6, more preferably at

20   least 7, at least 8, at least 9, or at least 10 signals of k is used. The relationship of formula (4) can be also used for all signals of k. Information on an orientation of the target protein or domain structures in the target protein against static magnetic field can

25   be obtained by diagonalizing the Saupe order matrix. Physical values obtained here are given as molecular orientation tensor quantity expressed by molecule coordinate axes. This molecular orientation tensor

quantity is expressed with molecular orientation intensity (orientation tensor which is a magnitude along each axial direction expressed in three-dimensional orthogonal coordinate system, and it is

5   quantity to express at what extent the molecule is oriented in the axis (which corresponds to existence probability)) and Euler angles (a, b, c) for the molecule coordinate of new coordinate axis (in which the Z-axis becomes a static magnetic field direction)

10   which describes the oriented state of the molecule. The formula above is illustratively shown with ^{15}N but it is not necessarily to use ^{15}N and other stable isotope (for example, ^{13}C) can be used.

[0119]

15   (Determination of information on an orientation of protein)

A protein structure is expressed by positions of atoms in x, y, z axes. When the protein is oriented against magnetic field, it is expressed to which extent

20   the coordinate axes (Dxx, Dyy, Dzz) expressing the protein in oriented state tilt from the original. For this purpose, three angles, α, β and γ shown in the drawings are defined. They are called Euler angles. The direction of orientation of the magnetic field is

25   expressed assuming that it agrees with the Dzz axis (Fig. 2).

[0120]

The index to express a change in the domain

structure includes (DOC) and degree of orientational similarity to the active conformation (DOS) in this specification, but it is not limited to these.

[0121]

5   In this specification, the "degree of orientational change" (DOC) between two orientations is given by, for example,

        (i) providing directions of orientation tensor axes by a first three unit vectors perpendicular

10   to each other, by using the first information on an orientation of the domain in the protein before the protein is bound/contacted with the compound, wherein the first three unit vectors are expressed by

[0122]

$$\vec{e}_{fx}, \quad \vec{e}_{fy}, \quad \vec{e}_{fz}$$

15   (ii) providing directions of orientation tensor axes by a second three unit vectors perpendicular to each other, by using the second information on an orientation of the domain in the protein after the protein is bound/contacted with the

20   compound, wherein the second three unit vectors are expressed by

[0123]

$$\vec{e}_{bx}, \quad \vec{e}_{by}, \quad \vec{e}_{bz}$$

(iii) denoting respective angles between the individual first unit vectors and the individual second unit vectors by a, b and c, and using the angles as an index to express change in the domain structure, or
5   alternatively giving a degree of orientational change by the following equation:

degree of orientational change = $a^2 + b^2 + c^2$;

and using this degree of orientational change as an index to compare the change in the domain
10   structure. For example, when these angles a, b and c are small or when DOC is small, it shows that the structural change of the protein induced by binding/contact of a compound is not significantly different from that in the absence of the compound.
15   Meanwhile, when these angles a, b and c are large or when DOC is large, it shows that the structural change of the protein induced by binding/contact of a compound is significantly different from that in the absence of the compound.
20   [0124]

When an agonist compound is screened, similarity of the structural change induced by binding of a ligand and the active structure can be used as an index. In this case, providing a third unit vectors
25   which express each axis of molecular orientation tensor defining domain orientation of active structure after binding/contacting with a ligand by

[0125]

$$\overrightarrow{e}_{ax}, \quad \overrightarrow{e}_{ay}, \quad \overrightarrow{e}_{az}$$

as well as using a second unit vectors after binding/contacting with a compound and denoting respective angles between the respective two unit

5   vectors by a, b and c, and using the angles as an index to express change in the domain structure, or alternatively giving a degree of orientational similarity to the active conformation (DOS) by the following equation:

10   degree of orientational similarity to the active conformation = $a^2 + b^2 + c^2$;

and using this degree of orientational change as an index to compare the change in the domain structure. For example, when these angles a, b and c

15 are small or when DOS is small, it shows that the structural change of the protein induced by binding/contact of a compound is close to the structural change of the protein induced by the ligand. That is, it can be an index showing similarity of the

20 structural change.

[0126]

In addition, the structural change of a domain in the protein is digitalized as difference of DOC and/or DOS based on the information on an

25 orientation in each state of the domain to be compared.

[0127]

Accordingly, when the change of the structure of the protein in the case that the first compound and the protein are contacted and the change of the structure of the protein in the case that the second compound and the protein are contacted are compared, "similarity" in the structural change of the protein means that the difference of DOS as the index of the structural change is small in this specification. For example, a second compound capable of inducing a structural change in a protein when it is contacted with the protein, similar to that induced in the protein when the first compound and the protein are contacted, can be selected as follows:

(a) selecting a domain in the protein;

(b) providing information on an orientation of the domain when the protein is in contact with the first compound;

(c) providing information on an orientation of the domain when the protein is in contact with the second compound, by

(i) providing known atomic coordinates for the domain,

(ii) providing axial variations of NMR signals, which are generated from the protein in contact with the second compound in the presence of a liquid crystalline material by two-dimensional TROSY NMR spectroscopy and depend on an orientation angle of

the molecules in a magnetic field,

(iii) determining Saupe order matrix elements for the domain from the atomic coordinates for the domain and the axial variations of NMR signals, and

5   (iv) diagonalizing the determined matrix to produce the information on an orientation of the domain; and

(d) comparing DOS between the atomic coordinates provided in step (b) and the atomic

10   coordinates provided in step (c).

[0128]

Therefore, in the case where the structural change caused by the first compound and the structural change caused by the second compound are identical,

15   $a=b=c=0$ and therefore, DOS $=0$. The case where the structural change caused by the first compound and the structural change caused by the second compound is "similar" includes cases where, for example, value of DOS is $75(\text{deg}^2)$ or less, $48(\text{deg}^2)$ or less, $27(\text{deg}^2)$ or

20   less, $12(\text{deg}^2)$ or less, $3(\text{deg}^2)$ or less, $0.75(\text{deg}^2)$ or less, $0.48(\text{deg}^2)$ or less, $0.27(\text{deg}^2)$ or less, $0.12(\text{deg}^2)$ or less or $0.03(\text{deg}^2)$ or less.

[0129]

Alternatively, whether the structural changes

25   are similar or not can be judged by, for example, calculating DOS between the second compound and the first compound in the group of plural candidate compounds and assuming that the second compound which

results in a relatively small DOS or the smallest DOS causes structural change which is similar to the structural change of the target protein caused by the first compound.

5 [0130]

(Acquisition of atomic coordinates of protein)

Group of M,Grestein of Yale University, USA collected the interdomain structure and function of a protein as a database named MolMovDB

10 (http://MolMovDB.org).

[0131]

For example, information on GroEL, adenylate kinase, calmodulin, HIV-1 reverse transcriptase, citrate synthase, diphtheria toxin, HIV-1 protease,

15 EGF-receptor, hexokinase, glucokinase, EPO receptor, phosophoglycerate kinase, recoverin, mRNA capping enzyme, DNA polymerase β are available from a database.

[0132]

(Method of obtaining information on an orientation of

20 protein domain from atomic coordinates of protein)

When atomic coordinates of a protein are obtained by the method mentioned above, information on an orientation of domains of the protein can be obtained from the atomic coordinates. For example, the

25 residual dipole effect D_{nh} can be expressed with an angle ϕ_i, angle of each NH bond spectrum in the main chain of a protein against the i-th (i=y, y, z) molecule coordinate axis and Saupe order matrix, S

defining orientation of molecule against the external magnetic field (S_{ij} represents an element of molecular orientation matrix; $i,j = x, y, z$) by the following relationship.

5   [0133]

$$D^k_{nh} = D^0_{nh} \Sigma\ S_{ij}\ \cos\ \phi^k_i\ \cos\ \phi^k_j \qquad (5)$$

$$i,j = x,y,z$$

Accordingly, every element S_{ij} of the Saupe order matrix that is necessary to define molecular

10 orientation can be determined from $\cos\ \phi^k_i\ \cos\ \phi^k_j$ calculated from atomic coordinates of domain of the protein, residual dipole effect D_{nh} between ^1H-^{15}N nuclear spins and intensity of residual dipole effect D^0_{nh} between ^1H-^{15}N.

15 [0134]

(Method of identifying a binding position on a protein when a compound has been bound to the protein)

When a compound has been bound to a protein, the binding position on the protein can be determined,

20 for example, according to a process as follows:

. providing a two-dimensional TROSY NMR spectrum of the protein in the absence of a compound,

. providing a two-dimensional TROSY NMR spectrum of the protein in contact with a compound,

25 . comparing the two-dimensional TROSY NMR spectra each provided above, and detecting a change of the spectra,

. identifying an amino acid residue

(preferably, more than one amino acid residue) around the site on the protein to which the compound is bound, by determining in which amino acid residue an atom that has produced a spectral change is contained by

5   assignment of the two dimensional NMR,

. if necessary, comparing the conformation of the compound with the conformation of the protein around amino acid residue specified above and estimating the conformation when the compound binds to

10   the binding site on the protein.

[0135]

(Observation of a structural change in a domain of a protein)

According to one aspect of the present

15   invention, structural change in a domain of the protein can be measured and/or a compound which results in structural change (preferably, desired structural change) can be selected, for example, by taking the following steps:

20   [0136]

(a) selecting a domain in the protein;

(b) providing information on an orientation of the domain when the protein is not in contact with the compound;

25   (c) providing information on an orientation of the domain when the protein is in contact with the compound, by

(i) providing known atomic coordinates for

the domain,

(ii) providing axial variations of NMR
signals, which are generated from the protein in
contact with the compound in the presence of a liquid
5 crystalline material by two-dimensional TROSY NMR
spectroscopy and depend on an orientation angle of the
molecules in a magnetic field,

(iii) determining Saupe order matrix elements
for the domain from the atomic coordinates for the
10 domain and the axial variations of NMR signals, and

(iv) diagonalizing the determined matrix to
produce the information on an orientation of the
domain; and

(d) measuring the structural change in the
15 protein by a difference between the atomic coordinates
provided in step (b) and the atomic coordinates
provided in step (c).

[0137]

As for domains to be observed by the process
20 of the present invention, any domain in the protein to
be observed is usable. However, for example, the
domain to be observed is preferably, for example, a
domain producing a large change in the information on
an orientation depending on the presence or absence of
25 the compound and/or a domain producing a different
change in the information on an orientation by binding
to a different compound.

[0138]

The above step (b) in the above measuring process may be, if necessary,

(b) providing the information on an orientation of the domain when the protein is not in contact with the compound, by

(v) providing known atomic coordinates for the domain,

(vi) providing axial variations of NMR signals, which are generated from the protein in no contact with the compound in the presence of the liquid crystalline material by two-dimensional TROSY NMR spectroscopy and depend on the orientation angle of the molecules in the magnetic field,

(vii) determining Saupe order matrix elements for the domain from the atomic coordinates for the domain and the axial variations of NMR signals, and

(viii) diagonalizing the determined matrix to produce the information on an orientation of the domain; or

(b) providing the information on an orientation of the domain from the atomic coordinates provided previously when the protein is not in contact with the compound. The TROSY spectrum to determine plural Saupe order matrix elements as above can be measured at the same time or separately.

[0139]

In addition, variations of NMR signal in the axial direction, which are generated in the above step

(b) and/or (c) by two dimensional TROSY NMR

spectroscopy and depend on the orientation angle of the

molecules in the magnetic field, may be variations in

the ^{15}N axial direction if ^{15}N is used. When the nuclide

5   of another stable isotope is used, it may be variations

in the axial direction of the nuclide.

[0140]

Here, when ^{15}N is used, the Saupe order matrix

elements in (iii) and/or (vii) are preferably

10   determined by:

with respect to the kth pair of ^{15}N nuclear

spins in the domain,

providing ϕ^k_i as a vector angle of an N-H bond

having the kth pair of spins in the domain to the ith

15   molecular axis,

providing ϕ^k_j as a vector angle of an N-H bond

having the kth pair of spins in the domain to the jth

molecular axis,

setting a static dipolar coupling D^0_{nh} at 23.0

20   kHz for a length of the N-H bond of 1.02 Å, or setting

a static dipolar coupling D^0_{nh} at 21.7 kHz for a length

of the N-H bond of 1.04 Å,

determining as δ_{ij} a tensor value for chemical

shift anisotropy of a ^{15}N nucleus in an arbitrary

25   residue of the protein, and

determining the Saupe order matrix elements

S_{ij} defining the molecular orientation with respect to

the magnetic field, by contacting the protein with the

compound in the presence of the liquid crystalline material, providing $\Delta\delta$trosy(k) for the kth pair of ^{15}N nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the $\Delta\delta$trosy(k) together with

5 the following equation (1):

$$\Delta\delta\text{trosy}(k) = \sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j + (2/3)\ \delta_{ij}\} \quad (1)$$

$$i,\ j = x,\ y,\ z$$

When a nuclide of stable isotope other than ^{15}N is used, the above calculation may be performed for

10 the spin pair of the nuclide. In that case, since the distance of the other different nuclides bond corresponding to the distance of N-H bond and static bipolar coupling are known, the known value can be used in the above equation.

15 [0141]

The structural change in a domain of a protein is provided by, for example, diagonalizing the Saupe order matrix elements to produce information on an orientation of the domain. Therefore, different

20 pieces of information on an orientation of the domain in itself can be compared to produce an index of a structural change in the domain. For example, when there is a change in information on an orientation of the predetermined domain between in the presence of and

25 in the absence of a compound, it turns out that the compound has brought about the structural change of the predetermined domain when it was bound to a protein. The information on an orientation in the presence of

and in the absence of a compound can be experimentally obtained respectively, but, for example, when information on an orientation under in the absence of the compound is already provided by NMR experiment or

5   X-ray analysis, the information can be used and compared with information on an orientation provided by experiment in the presence of the compound.
[0142]

        Furthermore, DOC and DOS can be used as an

10 index of the structural change. Therefore, DOC can be used as the size of the structural change that an arbitrary compound induces. When the value of DOC is large, it shows that the structural change is large. In addition, DOS can be used to find whether an

15 arbitrary compound produces a change of the structure similar to that of a certain ligand or agonist. When the value of DOS is smaller, it shows that the structural change resembles more.
[0143]

20   Screening of an agonistic agent which induces the target protein into activation can be performed, for example, by the following procedure:

        . providing information on an orientation (A) of domain in the target protein under the condition in

25 which a ligand or agonist known to induce structural change of domain binds to the target protein;

        . providing information on an orientation (B) of domain in the target protein under the condition in

which the candidate compound of screening binds to the target protein;

. comparing the information on an orientation (A) and (B) and determining whether the information on
5   an orientation is equivalence or similar.

. when these pieces of information on an orientation are equivalence or similar, it turns out that the candidate compound is a compound inducing a structural change of target protein which is similar to
10 that of an agonist inducing the target protein to be active. Therefore, the candidate compound selected in this way is a useful agonistic lead compound.
[0144]

In the present invention, a step for
15 specifying the position at which the compound binds to the protein can be performed if necessary. The specification of the position is performed, for example, by comparing the two dimension TROSY NMR spectrum provided in step (b) and the two dimension
20 TROSY NMR spectrum provided in step (c) to detect a change in the spectrum and identify the amino acid residue in the protein which has produced the change in the spectrum. Identification of this amino acid residue is performed based on the assignment of the
25 changed spectrum to the amino acid sequence.
[0145]

The liquid crystal material used in the present invention includes a mixture selected from the

group consisting the following:

a mixture of dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcholine (DHPC),

a mixture of dimyristoylphosphatidylcholine

5   (DMPC) and dihexanoylphosphatidylcholine (DHPC) and sodium dodecyl sulfate (SDS),

a mixture of dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcholine (DHPC) and cethyltrimethyl ammonium bromide (CTAB),

10   a mixture of 1,2-di-O-dodecyl-sn-glycero-3-phosphocholine (DIODPC) and 3-(cholamidepropyl)-dimethylammonio-2-hydroxy-1-propane sulfate (CHAPS),

a mixture of n-alkyl-poly (ethyleneglycol)/n-alkyl alcohol,

15   filamentous phage,

a mixture of cetyl pyridinium chloride (CPCl)-hexanol-NaCl

a mixture of cetyl pyridinium bromide (CPBr)-hexanol-NaCl,

20   purple membrane fragments of Halobacterium genus,

microcrystalline cellulose and

polyacrylamide gel (for example, acrylamide/acrylate gel) and a mixture of at least 2 of

25   the above mixtures.)

[0146]

For example, the liquid crystal material is a mixture of 7.5%(w/v) of dimyristoylphosphatidylcholine

(DMPC) and dihexanoylphosphatidylcholine (DHPC) and cethyltrimethyl ammonium bromide (CTAB).

[0147]

In another aspect of the present invention, a program carrying out the process of the present invention and a storage medium including such a program are also provided.

[0148]

In a still further aspect of the present invention, a device which can be used to carry out the process of the present invention and a device including the program of the present invention are provided.

[0149]

The present invention is described in detail below by way of Examples and the like, but the present invention is not limited to these.

Example 1

[0150]

(Orientation of protein by liquid crystal material)

Orientation of a protein by a liquid crystal material is performed by the following method, for example.

[0151]

(1. Weak orientation of a protein using a mixture of dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcholine (DHPC))

DMPC and DHPC are mixed preferably at a pH of 6.8 to 8.0, more preferably at pH 6.5, preferably at a

mixing ratio of 2.5 to 3.0, more preferably at a mixing
ratio 3.0. This mixture is used preferably at a
concentration of 5 to 20%(w/v), more preferably at a
concentration of 7.5%(w/v), and molecular orientation

5 is performed preferably at 25 to 40°C, more preferably
at 30°C (M.Ottinger and A.Bax, J.Biomol. Nuclear
magnetic resonance 12, 361 (1998)).

(2. Weak orientation of a protein using a mixture of
dimyristoylphosphatidylcholine (DMPC) and

10 dihexanoylphosphatidylcholine (DHPC) and sodium dodecyl
sulfate (SDS))

For example, a surfactant such as SDS is
added to a DMPC/DHPC solution of above 1 and the
surface of phospholipid membrane is negatively charged

15 to prevent unnecessary adsorption to phosphatide of a
protein having negative surface charge. When SDS is
added, mixing with a protein sample solution is
performed also preferably at a pH of 6.0 to 8.0, more
preferably at pH 6.5, preferably at a concentration of

20 5 to 20%(w/v), more preferably at a concentration of
7.5%(w/v), and molecular orientation is performed
preferably at 20 to 35°C, more preferably at 30°C. In
this case, DMPC and DHPC are mixed preferably at a
mixing ratio of 2.5 to 3.0, more preferably at a mixing

25 ratio 3.0. Preferably SDS is added to DMPC at a ratio
of 1 to 3%. (J.A.Losonczi and J.H.Prestigard,
J.Biomol. Nuclear magnetic resonance 12, 447 (1998))

(3. Weak orientation of a protein using a mixture of

dimyristoylphosphatidylcholine (DMPC) and

dihexanoylphosphatidylcholine (DHPC) and

cethyltrimethyl ammonium bromide (CTAB))

For example, a surfactant such as CTAB is

5 added to a DMPC/DHPC solution of above 1 to prevent

unnecessary adsorption to phosphatide of a protein

having positive surface charge. When CTAB is added,

mixing with a protein sample solution is performed also

preferably at a pH of 6.0 to 8.0, more preferably at pH

10 6.5, preferably at a concentration of 5 to 20%(w/v),

more preferably at a concentration of 7.5%(w/v), and

molecular orientation is performed preferably at 20 to

35°C, more preferably at 30°C. In this case, DMPC and

DHPC are mixed preferably at a mixing ratio of 2.5 to

15 3.0, more preferably at a mixing ratio 3.0. Preferably

CTAB is added to DMPC at a ratio of 1 to 3%.

(J.A.Losonczi and J.H.Prestigard, J.Biomol. Nuclear

magnetic resonance 12, 447 (1998))

(4. Weak orientation of a protein using a mixture of

20 1,2-di-O-dodecyl-sn-glycero-3-phosphocholine (DIODPC)

and 3-(cholamidepropyl)-dimethylammonio-2-hydroxy-1-

propane sulfate (CHAPS))

The ratio of DIODPC/CHAPSO is most

appropriately 4.8, but it is not limited to this. The

25 concentration of phospholipid membrane in the sample is

most appropriately 5%(w/v), but it is not limited to

this. Preferably pH of the sample solution is 1.0 to

6.5. Molecular orientation is preferably in the range

of 10 to 60°C (S.Cavagnero et al., J.Biomol.NMR 13, 387
(1999)).

[0152]

(5. Weak orientation of a protein using a mixture of n-
alkyl-poly(ethyleneglycol)/n-alkylalcohol)

The alcohol mixture mentioned above is not
limited, but molecular orientation can be induced
preferably by adding 5%(w/v) to the solution in the
range of 0 to 40°C. As for the kind of alcohol, n-
hexanol or n-octanol can be used. The ratio of n-
alkyl-poly(ethyleneglycol)/n-alkylalcohol is preferably
in the range of 0.6-0.9. As for pH, any pH can be
used. (M. Ruckert and G. Otting, J. Am.Chem.Soc.122,
7793 (2000))

(6. Weak orientation of a protein using filamentous
phage)

Molecular orientation in the magnetic field
can be realized by letting a bacteriophage having a
fibrous shape such as pf1 or tobacco mosaic virus (TMV)
coexist in the sample solution. Preferably
bacteriophage concentration in the sample solution is
in the range of 10 to 50 mg/ml. Preferably the
concentration is 25 mg/ml. Preferably pH is equal to
or less than 6. Any temperature can be used, but
preferably it is used in the range of 20 to 40°C.
(Hansen et al., Nat.Struct.Biol. 5, 1,065 (1998); Clore
et al., J.Am.Chem.Soc. 120, 10,571 (1998))

(7. Weak orientation of a protein using a cetyl

pyridinium chloride (CPCl)-hexanol-NaCl mixture or cetyl pyridinium bromide (CPBr)-hexanol-NaCl mixture)

CPBr: hexanol =1:1.33(w/w) to 1:1(w/w) is used. The concentration of NaBr is preferably 25 to 40

5   mM, and the concentration of NaCl is preferably 200-500 mM. The concentration when this liquid crystal material is used is preferably 1 to 10%(w/v), and molecular orientation is preferably performed at a temperature in the range of 15 to 50°C.

10   [0153]

(8. Weak orientation of a protein using a purple membrane piece of genus Halobacterium)

A purple membrane piece of genus Halobacterium, preferably a purple membrane piece of

15   Halobacterium salinarium is used at a concentration of 1 to 10 mg/ml. Any solution temperature and any pH can be used. Preferably NaCl concentration in the solution is 50 mM or less. (Prosser et al., J.Am.Chem.Soc.120, 11010 (1988))

20   (9. Weak orientation of a protein using microcrystalline cellulose)

Preferably a solution of 80 mg/ml is prepared and molecular orientation is possible by introducing a protein. Any pH and temperature can be used. When the

25   ion concentration of the solution increases, the sample solution which is necessary to realize molecular orientation is added. (Fleming et al., J.Am.Chem.Soc.122, 5224 (2000))

(10. Weak orientation of a protein using polyacrylamide
gel)

A protein solution is injected into
polyacrylamide gel which has been compressed in
5 longitudinal or lateral direction in a NMR sample tube
and a protein is automatically subjected to molecular
orientation. Any pH, ionic strength and observation
temperature can be used. (Saas et al., J.Biomol.NMR
18, 303 (2000); Tycko et al., J.Am.Chem.Soc. 122, 9340
10 (2000))

(11. Orientation of a protein using acrylamide/acrylate
gel)

As an example of polyacrylamide gel, a gel as
alignment medium may be used which is prepared by
15 stretching 50% acrylamide/50% acrylate gel by 2.5
times. When the acrylamide/acrylate is gelled, a
labeled protein may be added to orient the protein in
the gel.

Example 2

20 [0154]

(Comparison of information on a molecular orientation
obtained from TROSY signal change with the results of
conventional analysis)

0.5 mM ubiquitin (8.6 kDa) was dissolved in a
25 20 mM sodium phosphate buffer solution (pH 6.4) and
7.5%(w/v) of DMPC/DHPC/CTAB was added to form a
molecular orientation state at 30°C and the solution was
tested by the following procedure.

[0155]

(Experiment)

(NMR spectroscopy)

All the spectra used by this analysis were recorded at 30°C under the condition in which a bicelle mixture of dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcholine (DHPC) doped with cethyltrimethyl ammonium bromide (CTAB) was arranged so that it gave 10 Hz of residual quadrupole splitting in ^{2}HNMR spectrum. Data acquisition was carried out on the Varian INOVA500 spectroscope with ^{1}H frequency operated at 499.84 MHz. The present inventors used ^{1}H detection coil Varian gHX NanoProbe equipped with X adjusted to ^{15}N for magic angle test. Sample rotating speed was set to 2.6 kHz and the torque caused by having arranged bicelle with regard to the magnetic field was removed (Courtieu,J., Bayles,J.P.; Fung,B.M., Prog. NMR spectrosc.1994,26,141-169; Kurita,J., Shimahara,H. Utsunomiya-Tate,N.; Tate,S. J.MagnReson.2003,163,163-173.). ^{1}H-^{15}N dipole coupling was measured in ^{1}H-^{15}N IRAP-HSQC spectrum (Ottiger,M.; Delaglio,F.; Bax,A. J.Magn Reson.1998,131,373-378.). ^{1}H-^{15}N TROSY spectrum (Pervushin,K.: Riek,R.; Wider,G.; Wuthrich,K. Proc.Natl.Acad.Sci.U.S.A1997,94,12366-12371, Weigelt,J.J.Am.Chem.Soc.1998,120,10778-10779.) was collected in both isotropic state and anisotropic state using a solution of 50 mM sodium phosphate (pH 6.4) containing 7.5% (mass/volume) of DMPC:DHPC:CTAB bicelle

and the same solution containing 0.5 mM ^{15}N labeled

ubiquitin dissolved in 2 mM EDTA as sample solutions

without using magic angle sample rotation method.

[0156]

5   All the data was processed using NMRpipe

program software package (Delaglio,F.; Grzesiek,S.;

Vuister G.W.; Zhu,G.; Pfeifer,J.;Bax,A.J. Biomol. NMR

1995,6,277-293.). Peak position was determined by

fitting an ellipse to each of the calculated contour

10 between 60% and 80% of the peak maximum using program

PIPP(Garrett,D.S.; Powers,R.; Gronenborm,A.M.;

Clore,G.M,J. Magn. Reson.1991,95,214-220.). The center

of each ellipse provides criterion of peak position and

values obtained for all contours in the intensity range

15 of 60 to 80% of the predetermined peak were averaged so

as to provide peak position. Acquisition time data for

acquisition at t_1 dimension and at t_2 dimension was 111

ms and 128 ms respectively and had a matrix size of 200

(t_1, ^{15}N) × 1024 (t_2, ^{1}H) complex points. The final

20 processed data widely zero-filled showed digital

resolution of 2.0 Hz and 0.88 Hz for ^{1}H dimension and

^{15}N dimension respectively.

[0157]

(Calculation of sequence tensor)

25   Determination of sequence inductive TROSY

shift change and lining-up tensor derived from error

estimation was carried out by in-house C-program

incorporated into a public subroutine (Press,W.H.;

Teukolsky,S.A.; Vetterling,W.T.; Flannery,B.P.
Numerical recipes in C 2nd ed.; Cambridge university
press: New York, 1992). Sequence tensor was calculate
from experimental data, and calculated from reference

5 structure generated by 1.8 Å X-ray coordinate (PDB
entry code, 1UBQ) (Vijay-Kumar,S. Bugg,C.E.;
Cook,W.J.J.Mol Biol. 1987,194,531-544.) having a
hydrogen atom added by program MOLMOL (Koradi,R.;
Billeter,M.; Wuthrich, K.J.Mol.Graph.1996,14,51-55.).

10 The effect of TROSY shift on experimental indeterminacy
was evaluated by repeating specific value decomposition
(SVD) calculation (Losonczi,J.A.; Andrec,M.;
Fischer,M.W.; Prestegard,J.H.J.Magn.Reson.1999,138,334-
342.) on the generated data set. This data set was

15 generated by adding Gaussian noise to the experimental
data. Gaussian noise was assumed to distribute in a
relative probability $(-\exp(x^2/2\delta^2))$. Here, x is an
experimental value, and δ is a rms noise estimated from
rms deviation of TROSY shift during continuous

20 experiments.

[0158]

"Structural noise" was simulated by
reorienting NH binding vector in a random manner by a
method similar to that described in reference

25 (Zweckstetter,M.; Bax,A. J.Biomol. Nuclear magnetic
resonance, 2002,23, 127-137.). The deviation between
the original vector and the reoriented vector was
described with a circular cone having 5 degrees slope

angle from the original direction. Slope angle between

the original NH vector and the reoriented NH vector is

assumed to be in Gaussian distribution $(-\exp(\alpha^2/2\theta^2))$ in

the simulation of this structure noise. Here, α is a

5   slope angle and θ is an estimated standard deviation for

a slope angle fixed to five degrees. The angle of

rotation in the lateral direction of vector reoriented

in the circular cone was simulated assuming that it was

uniformly distributed. Selection of appropriate values

10   from the simulated data was performed by maintaining

the rms deviation between the experimental data and

simulated $\Delta\delta_{trosy}$ value to less than 2.55 Hz. This value

shows a rate accepted for 68% of the simulated values,

and this value means that these values are present

15   within one standard deviation of average.

[0159]

        $\Delta\delta_{trosy}$ values for ^{15}N labeled ubiquitin

dissolved in 7.5%(w/v) DMPC/DHPC/CTAB bicelle at 30°C

are shown in the following Table.

[0160]

[Table 1]

Residue	$\Delta\delta_{trosy}$/Hz	Residue	$\Delta\delta_{trosy}$/Hz
13 ILE	0.64	45 PHE	-0.23
14 THR	3.01	47 GLY	2.59
15 LEU	1.54	48 LYS	-5.99
16 GLU	1.86	49 GLN	-2.63
17 VAL	-0.76	50 LEU	0.31
18 GLU	-3.08	51 GLU	-2.01
20 SER	-2.47	54 ARG	2.75
21 ASP	4.81	55 THR	0.23
23 ILE	0.04	56 LEU	1.72
25 ASN	-1.66	57 SER	5.55
26 VAL	0.97	58 ASP	1.02
27 LYS	0.66	59 TYR	2.36
28 ALA	0.82	60 ASN	4.69
29 LYS	0.04	61 ILE	5.74
30 ILE	2.33	62 GLN	6.21
31 GLN	2.40	63 LYS	-5.80
32 ASP	-1.96	64 GLU	2.27
33 LYS	0.77	65 SER	6.61
34 GLU	1.52	66 THR	4.90
35 GLY	-0.54	67 LEU	4.34
36 ILE	-3.50	68 HIS	0.54
39 ASP	3.12	69 LEU	1.09
40 GLN	-2.63	70 VAL	2.00
42 ARG	4.90	71 LEU	2.01
44 ILE	2.05	72 ARG	4.93

In the experiment which gave the above results, experiment was performed without applying MAS or by applying MAS respectively for obtaining data collected in an anisotropic state and aligned state.

5　MAS (magic angle sample spinning) is an abbreviation of magic angle sample spinning. When NMR measurement is performed with MAS applied, molecular orientation can be physically cancelled even if a liquid crystal material coexists. Therefore the same data in the case

10　where a liquid crystal material does not coexist can be obtained even if a liquid crystal material coexists.

[0161]

$\Delta\delta_{trosy}$ value was calculated from a series of $^1H-^{15}N$ TROSY data by

$\Delta\delta_{trosy}$ value = $\delta^{15}N_{trosy}$ (MAS not applied) −

5   $\delta^{15}N_{trosy}$(MAS applied)

[0162]

Determination of Saupe order matrix element was performed as follows.

[0163]

10   With respect to the kth pair of ^{15}N nuclear spins in the domain,

providing ϕ^k_i as a vector angle of an N−H bond having the kth pair of spins in the domain to the ith molecular axis,

15   providing ϕ^k_j as a vector angle of an N−H bond having the kth pair of spins in the domain to the jth molecular axis,

setting a static dipolar coupling D^0_{nh} at 23.0 kHz for a length of the N−H bond of 1.02 Å, or setting

20   a static dipolar coupling D^0_{nh} at 21.7 kHz for a length of the N−H bond of 1.04 Å

determining as δ_{ij} a tensor value for chemical shift anisotropy of a ^{15}N nucleus in an arbitrary residue of the protein, and

25   determining the Saupe order matrix elements S_{ij} defining the molecular orientation with respect to the magnetic field, without contacting the protein with the compound in the presence of the liquid crystalline

material, providing Δδtrosy(k) for the kth pair of ^{15}N nuclear spins of the protein by two-dimensional TROSY NMR spectroscopy, and using the Δδtrosy(k) together with the following equation (1):

5 $$\Delta\delta trosy(k) = \sum S_{ij}\{0.5\ D^0_{nh}\ \cos\phi^k_i\ \cos\phi^k_j + (2/3) \delta_{ij}\} \quad (6)$$

$$i,\ j = x,\ y,\ z$$

[0164]

Euler angles α, β, γ were obtained by

10 diagonalizing this S.

[0165]

The molecular orientation angle of ubiquitin determined from TROSY signal change induced by this molecular orientation was obtained from conventional

15 residual dipole effect observation.

[0166]

The residual dipole effect observation was performed by observing the intensity change shown by ^1Jnh (single bond spin coupling constant between ^1H-^{15}N

20 nuclear spins) of signals derived from each amino group which are observed before and after inducing molecular orientation by using liquid crystal molecules following a conventional residual dipole effect observation method: IPAP-HSQC method (Inphase-Antiphase mode

25 Heteronuclear Single Quantum Coherence spectroscopy; Ottiger et al., J.Magn.Reson.131,373(1998)) and calculating the residual dipole effect D_{nh} between each of the ^1H-^{15}N nuclear spins using ^1Jnh (aniso) observed

in the molecular orientation state with liquid crystal
and ^1Jnh (iso) observed when it is not in the molecular
orientation state without liquid crystal, in the
following equation:

5          $$D_{nh} = {}^1Jnh \ (aniso) - {}^1Jnh \ (iso)$$

[0167]

        The results of having compared it with
molecular orientation angle provided from residual
dipole effect observation are as follows. (tilt of
10 molecular orientation axis with regard to the molecule
coordinate axis is shown in Euler angles expression).
[0168]

[Table 2]

Process	Euler angle		
	α/deg.	β/deg.	γ/deg.
The inventive technique	70.6 ± 3.8	69.7 ± 2.1	78.7 ± 2.3
Prior Art	73.1 ± 1.3	70.3 ± 1.0	78.9 ± 1.1

        From these results, it was confirmed that
molecular orientation angle information determined
15 based on a TROSY signal change gave results which are
consistent to conventional molecular orientation
determination technique. In other words, it has been
shown for high molecular weight protein that the
structural change of the target protein induced by
20 binding to a compound can be quantitatively observed

only by a TROSY signal change.

Example 3

[0169]

(Observation of structural change for high molecular
weight protein using the present invention)

Information on an orientation of protein
having molecular weight more than 30 kDa which was not
able to measure by conventional NMR was obtained by the
present invention and a structural change was measured
as follows.

[0170]

As an object protein, mRNA5'-capping enzyme
(PBCV1-CE) which is a guanylyl transferase of chlorella
virus PBCV1 having molecular weight of 35 kDa was used.
Molecular weight of 35 kDa is a molecular weight region
in which it becomes difficult to analyze molecular
orientation by conventional measuring methods. It is
well known that activity is controlled in this protein
depending on a change of conformation induced by a
ligand. It has been made clear that PBCV1-CE which is
not bound to a ligand takes two structures (an open
type and a closed type) in the crystal by
crystallography. In this example, structure in a
solution of PBCV1-CE in the coexistence of
pyrophosphoric acid which is binding inhibitor of GTP
which induces an active form structure was analyzed and
a change from the structure which did not have a ligand
was analyzed.

[0171]

(Assignment of main chain)

Because assignment data of main chain was necessary for data analysis of TROSY measurements, 5 about 50% of the main chain was assigned using PBCV1-CE triplexly labeled with $^2H/^{13}C/^{15}N$.

(Conditions for TROSY measurements)

PBCV1-CE was oriented with regard to the magnetic field by putting 0.5 mM PBCV1-CE (200 mM 10 potassium phosphate, pH 7.6 labeled with $^2H/^{15}N$ into a 6%(w/w) gel of 50% acrylamide/50% acrylate (alignment medium) (Fig. 3) stretched by about 2.5 times. 5 mM pyrophosphoric acid, an activity inhibitor, was added to the sample solution. TROSY measurement was 15 performed at 20°C (Fig. 4). TROSY shift change dependent on molecular orientation was observed as a deviation from the TROSY signal in a state of normal solution to ^{15}N axis side for a TROSY signal of PBCV1-CE in the alignment medium. Based on a TROSY shift change 20 which showed sufficient separation of signals for 51 residues (N-terminal domain, 23 residues, C-terminal domain, 28 residues) and orientation tensor of each domain was determined.

Analysis was performed based on the 25 structures having two different domain orientations of PBCV1-CE (open type and closed type) which were found in crystal structure to confirm accuracy of the results of provided relative domain orientation. Two residues

of N terminal domain were removed from analysis in order to exclude influence of structural change in partial domain in the analysis based on crystal structure of closed type.

5 (Molecular orientation analysis)

According to the process of the present invention, molecular orientation between domains was analyzed with data of open type structure. $\Delta\delta$trosy value was obtained and Saupe order matrix elements were 10 determined similarly as in Example 2 and Euler angles α, β and γ in the absence of nucleotide were determined.

PBCV1-CE has a clear domain structure in its conformation. Orientation tensor of each domain was determined from a change of the TROSY signal which is 15 dependent on orientation and respectively derived from each of the domains (small domain) comprising 239-311 as well as a domain (large domain) comprising 15-222 with an amino acid residue number. As a result, as show in table 3, the Euler angles which express a tilt 20 of the molecular orientation axis from the molecule coordinate axis are (α =95.9°, β =123.1°,γ =96.2°, Q-factor=0.38) for a large domain (N-terminal) and (α =98.4°, β =114.7°,γ =165.4°, Q-factor=0.45) for a little domain (C-terminal).

[Table 3]

	Euler angles[°]			Q-factor
	α	β	γ	
N-terminal	95.9	123.1	96.2	0.38
C-terminal	98.4	114.7	165.4	0.45

Molecular orientation axes of each domain are as shown in Fig. 5 and Fig. 6. The relative orientation of domain was determined by rotating the small domain so that two orientation axes agree with each other, and it became clear that the small domain has rotated at about 65° compared with the crystal structure from orientation tensor of each domain.

Structure which had another domain orientation (closed type) of PBCV1-CE found in crystal structure was used to confirm the accuracy of the results of relative domain orientation provided by using an open type structure. The results are shown in the following Table 4 and Fig. 7 and Fig. 8.

[Table 4]

	Euler angles[°]			Q-factor
	α	β	γ	
N-terminal	77.2	124.3	87.1	0.46
C-terminal	105.4	115.2	150.3	0.42

The relative orientations between domains determined as a result of method of analysis of the

present invention are similar for open type and closed type and, therefore, accuracy of relative orientation between determined domains was confirmed.

[0172]

Using a similar method, Euler angles α, β and γ are determined in the presence of GTP and in the presence of ATP, respectively. Furthermore, from this Euler angles, DOCs at the time of GTP addition and ATP addition were calculated. These DOCs are an index of structural change at the time of GTP addition and at the time of ATP addition.

Example 4

[0173]

(Screening of agonistic lead compounds for a protein)

Search for a new agonist and/or antagonist of protein is important for the production of a new drug. Particularly, a new agonist could be used as an agonistic lead compound, but the conventional technique was inadequate for identification of agonistic lead compounds.

[0174]

Therefore, screening of agonistic lead compound is performed according to the present invention. mRNA5'-capping enzyme used in Example 3 is used as a model.

[0175]

Since it is believed that an agonistic lead compound produces a structural change which is similar

to GTP activating mRNA5'-capping enzyme in mRNA5'-capping enzyme, screening is performed as follows.

[0176]

Information on an orientation of mRNA5'-capping enzyme is obtained under a similar condition as in Example 3 except that an experiment is performed in which one of the candidate compounds is substituted for GTP. Nucleotide analogs, particularly GTP analogs and ATP analogs can be used as a candidate compound. DOS was calculated from information on an orientation in the presence of GTP and information on an orientation in the presence of the candidate compound.

[0177]

A compound having small DOS value is selected as an agonistic lead compound from plural candidate compounds.

Example 5

[0178]

(Identification of binding site of an agonistic lead compound selected from candidate compounds)

When an agonistic lead compound has been selected, binding site of the agonistic lead compound can be identified. In this example, a compound which produced the smallest DOS value in Example 4 is used and experiment is performed as follows.

. Performing assignment of two-dimensional TROSY NMR spectrum to amino acid residues in the absence of the candidate compound used in Example 4

according to a conventional process.

. Comparing two dimensional TROSY NMR spectrum in a domain of the protein in the presence of the candidate compound and two dimensional TROSY NMR

5 spectrum in the absence of the candidate compound. A spectral change is detected from the comparison. An amino acid residue corresponding to the changed spectrum is identified as a binding site of the agonistic lead compound from the results of the above

10 assignment.

Example 6

[0179]

(Determination of affinity of compound to target protein)

15 In the present invention, affinity of a compound to the target protein can be determined, for example, by the following method.

. Assignment of two-dimensional TROSY NMR spectrum to amino acid residues in the absence of a

20 candidate compound used in Example 4 is carried out according to a conventional process.

. Two dimensional TROSY NMR spectrum in a domain of the protein in the presence of the compound and two dimensional TROSY NMR spectrum in the absence

25 of the compound are compared. A spectral change is detected by the comparison. An amino acid residue corresponding to the spectral change is identified from the results of the above assignment.

. The spectrum corresponding to the
identified amino acid appears in different positions in
the absence of the compound and in the presence of the
compound. However, there is a case where the spectrum

5 corresponding to the identified amino acid detected in
the absence of a compound remains even in the presence
of the compound depending on the type of the compound.
The reason why the spectrum corresponding to a single
amino acid residue doubly exists is that a compound

10 bound to the protein and a compound not bound to the
protein are both present in the solution of two-
dimensional TROSY NMR measurement. The ratio of
intensity of spectrum corresponding to this doubly
existing single amino acid residue relates to the

15 abundance ratio with compound binding protein and
compound non-binding protein. Therefore, the ratio of
intensity of this double spectrum is an index of
affinity of a compound tested and the protein. Based
on this principle, the relative affinity of the

20 compound can be measured as follows:

. identifying the amino acid residue which a
compound binds following Example 5;

. performing the NMR measurement in the
presence of Compound A and with regard to the spectrum

25 corresponding to the amino acid to which the compound
binds, calculating the ratio of (spectrum appearing at
a position detected only in the presence of Compound
A)/(spectrum which remains at a position detected in

the absence of compound);

    . performing the NMR measurement in the presence of Compound B and with regard to the spectrum corresponding to the amino acid to which the compound

5 binds, calculating the ratio of (spectrum appearing at a position detected only in the presence of Compound B)/(spectrum which remains at a position detected in the absence of compound); and

    . comparing these ratios for Compounds A and

10 B, and judging that a compound having a larger ratio has a larger affinity to the target protein.

INDUSTRIAL APPLICABILITY

[0180]

    Screening of ligand, agonist, antagonist of a

15 protein becomes rapid and easy by detecting a structural change of the protein occurring by binding of a compound. Furthermore, detection of structural change enables to screen a candidate compound of a new drug using as an index whether an active structure of

20 the target protein is induced or not.